MATE
MAGYAR AGRÁR- ÉS
ÉLETTUDOMÁNYI EGYETEM

# Traditonak fish genetics and the genetic markers

Dr. Balázs Kovács

# Genetics background of fish

- Fishes are the most diverse group of vertebrates
- 30,000 different species.
- Tree evolutionary different groups
    - Agnatha (hagfish, lamprey)
    - Chondrichthyes (cartilaginous fishes – sharks, rays)
    - Osteichthyes (Teleost fishes - carp, zebrafish)

# Genome structure 1.

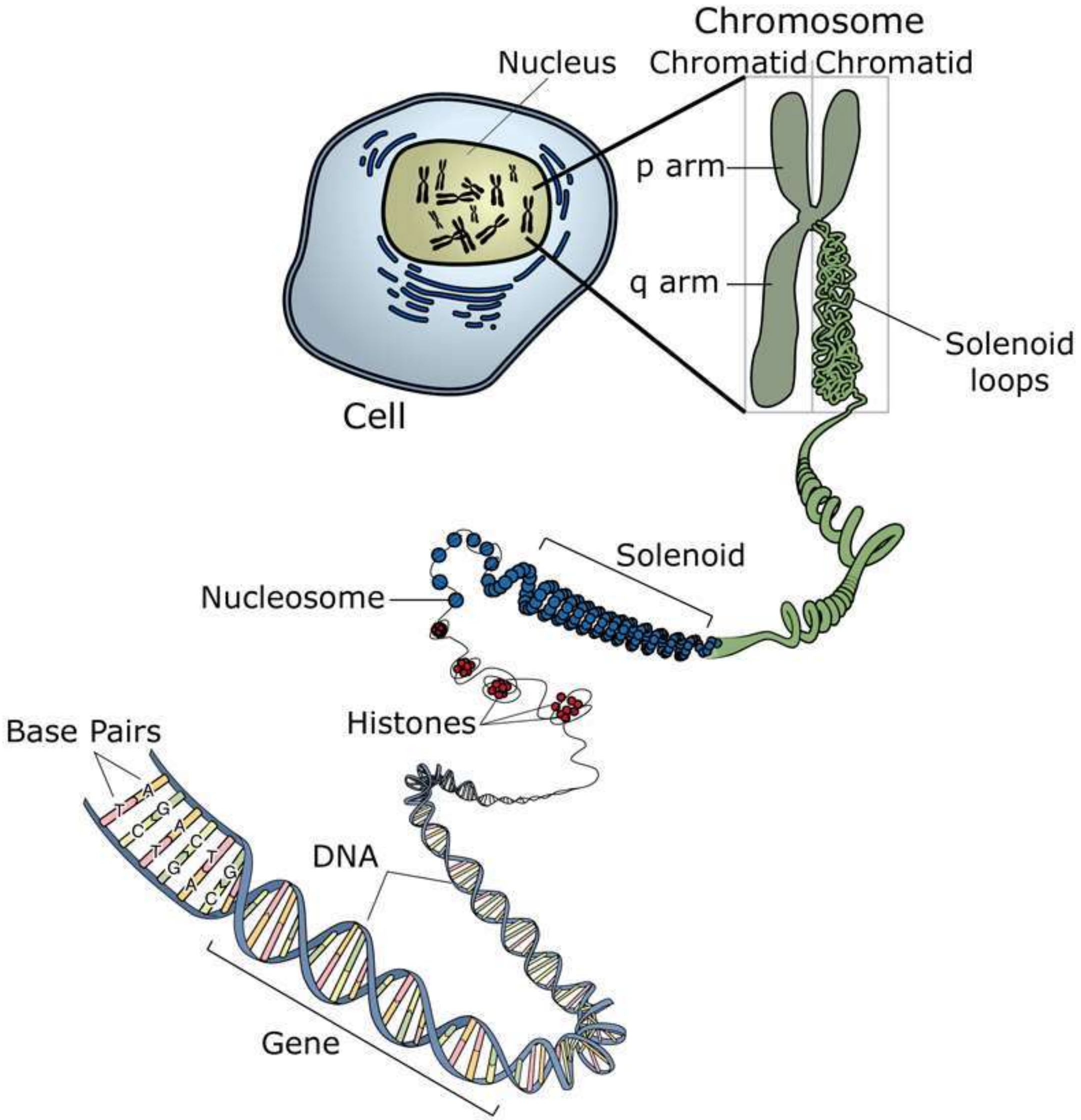


Image adapted from: National Human Genome Research Institute.

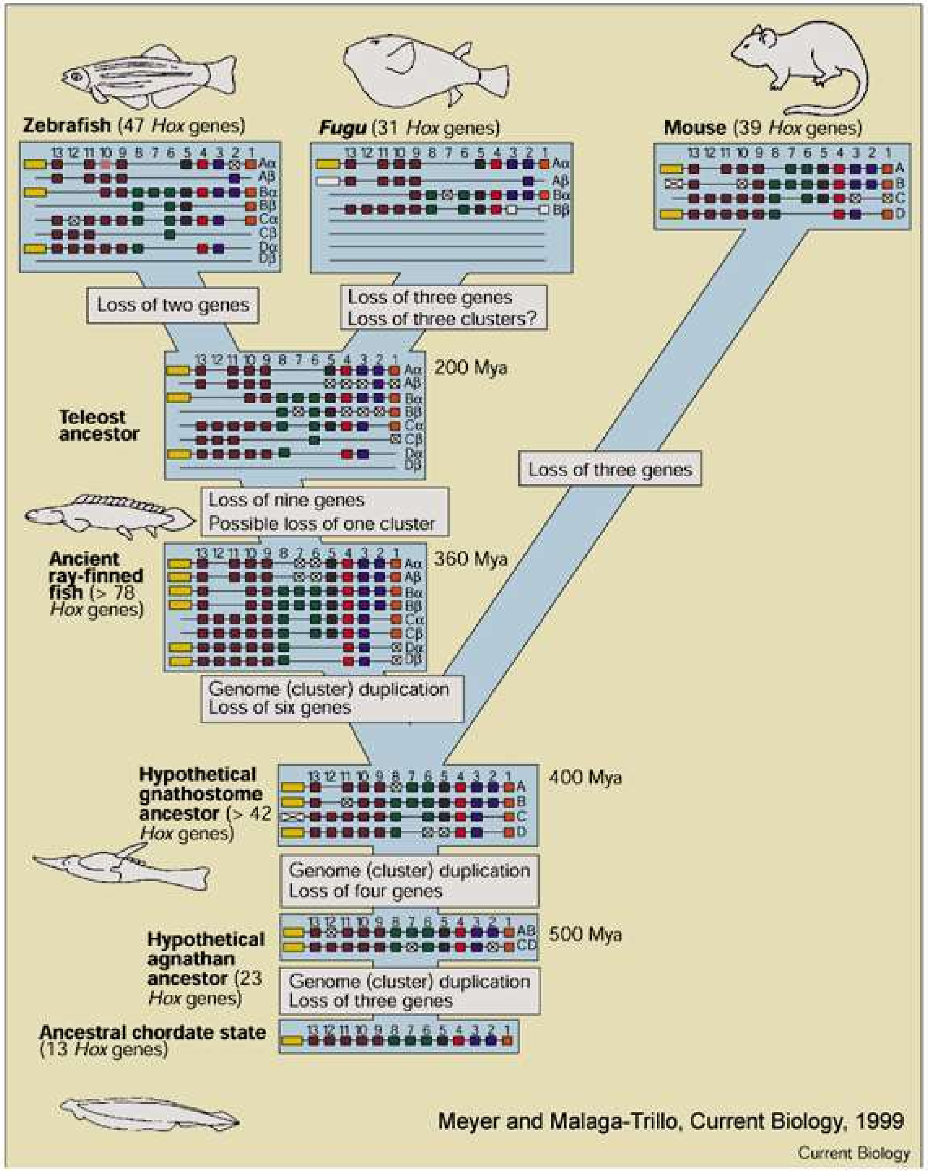
# Genome structure 3.

- One nucleus per cell
- Two chromosome set per cell
- Replication with cell reproduction
- Mendelian inheritance
- recombination
- Big size / milliards bp
- ~30 thousand genes
- ~1-3% coding region
- ~1-2% regulating region

# Genetics background of fish

More genes in fish?

Changes of Hox gene clusters and gene numbers
During the evolution.

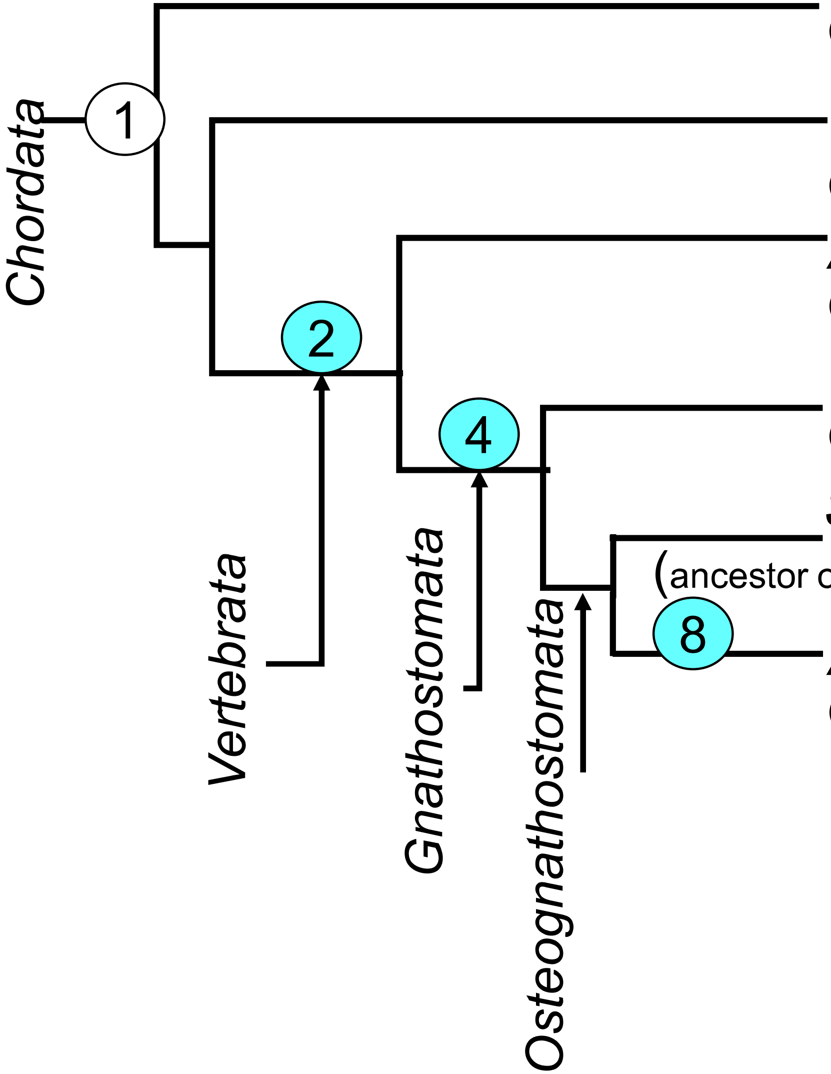


Meyer and Malaga-Trillo, Current Biology, 1999

Current Biology

# Genetics background of fish

GENOM



Chordata

Vertebrata

Gnathostomata

Osteognathostomata

*Urochordata*
(sea squirts, and sea pork)

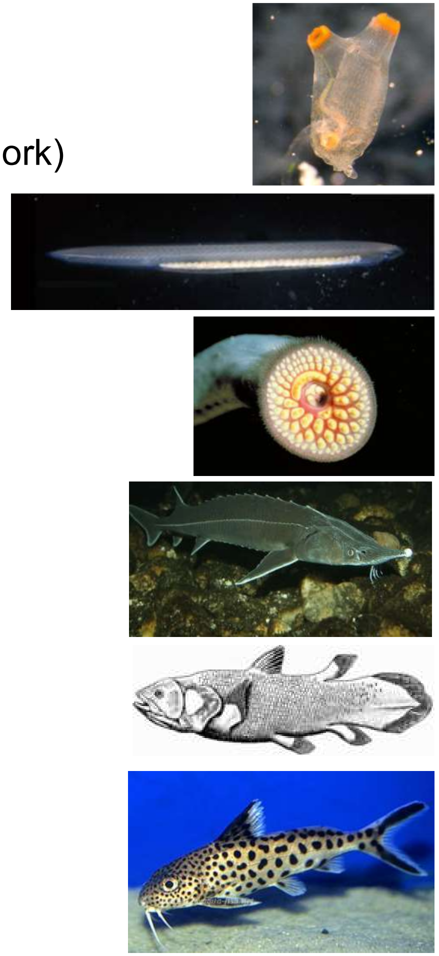
*Cyclostomatida*
(Lancelets)

*Agnatha*
(hagfish, lamprey)

*Chondrichthyes*
(sharks, ray)

*Sarcopterygii*
(ancestor of tetrapod, latimeria)

*Actinopterygii*
(ray-finned fishes
zebrafish, Carp,
Catfish, etc.)



# Genome size　1.

Smallest animal genome size:0.02pg

Plant-parasitic　nematode

(*Pratylenchus coffeae*),

Human genome size: 3.5pg

(*Homo sapiens*)

# Genome size 2.

Smallest vertebrate and fish genome size: 0.35pg

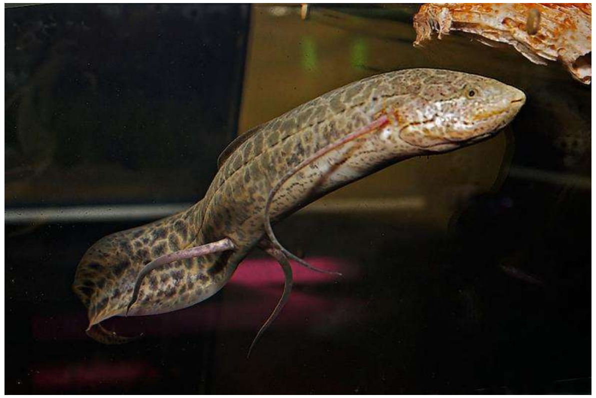
Green puffer fish

(*Tetraodon fluviatilis*)

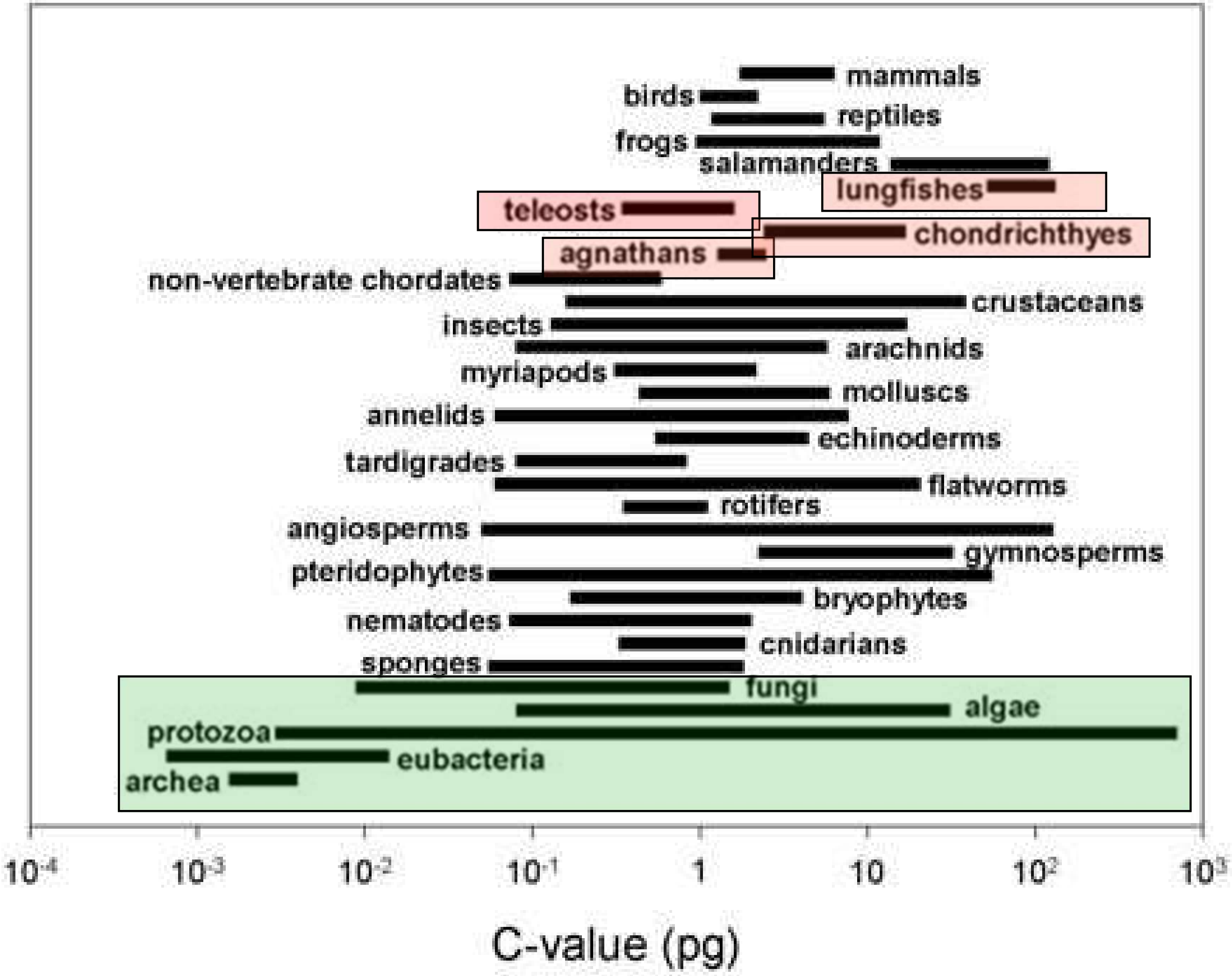
Largest fish and animal genome size:132.83pg

Marbled lungfish

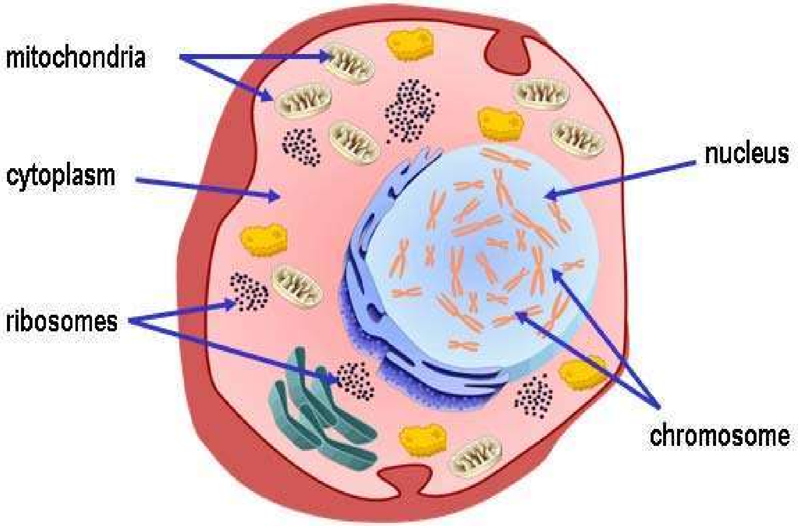
(*Protopterus aethiopicus*)

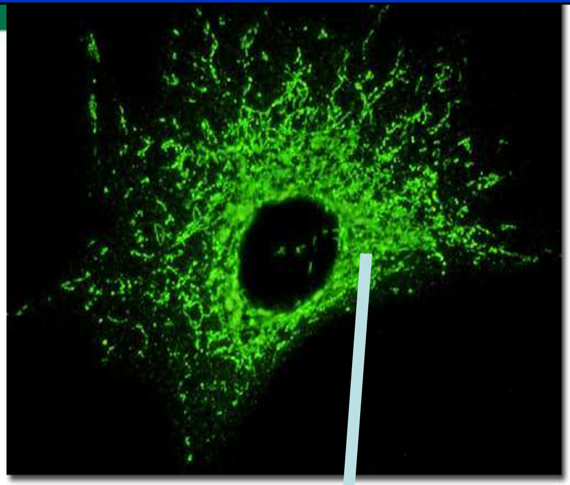


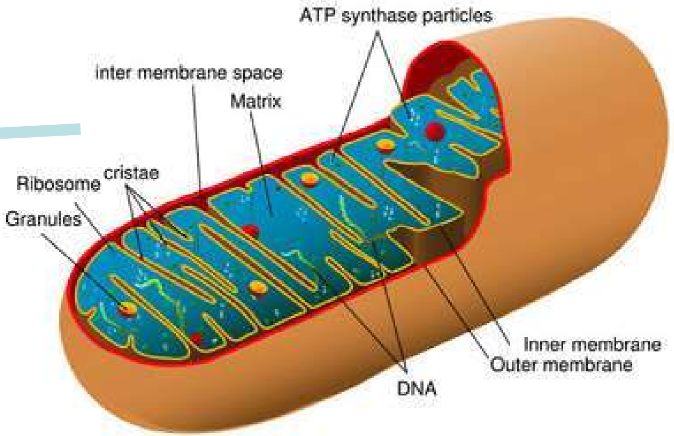
# Genome size 3.

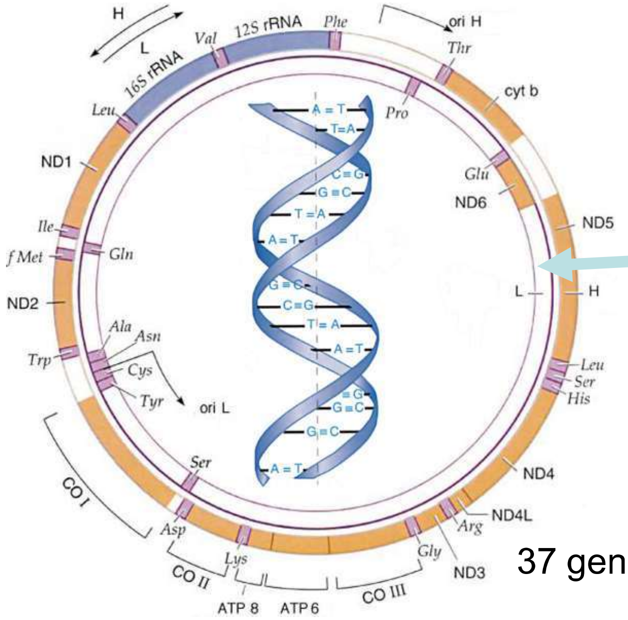


# Mitochondrial DNA 1.







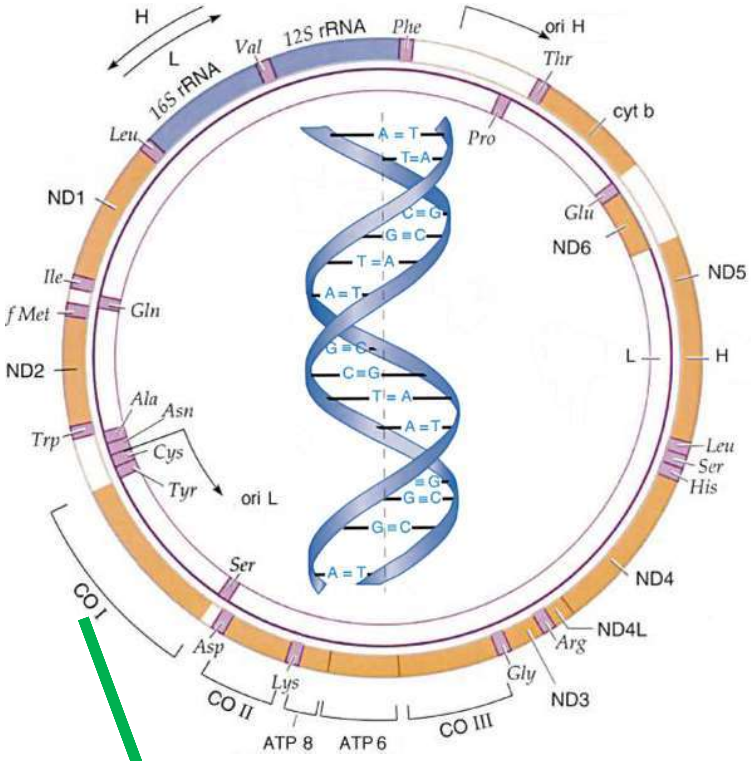


37 genes

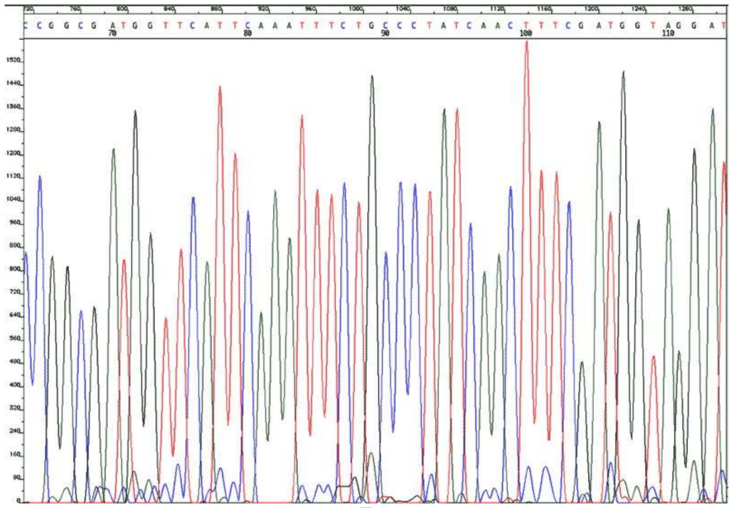
# Mitochondrial DNA 2.

- More thousand mitochondria per cell
- Independent replication from the nucleus
- Circular molecule
- Maternal inheritance
- No recombination
- Small size/16-17 thousand bp (1% of a bacterial genome)
- 37  gene

# Barcoding 1.



sequencing

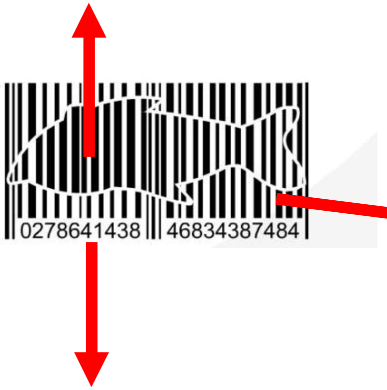


Species identification

Cytochrome oxidase I

# Barcoding 2.

Species identification

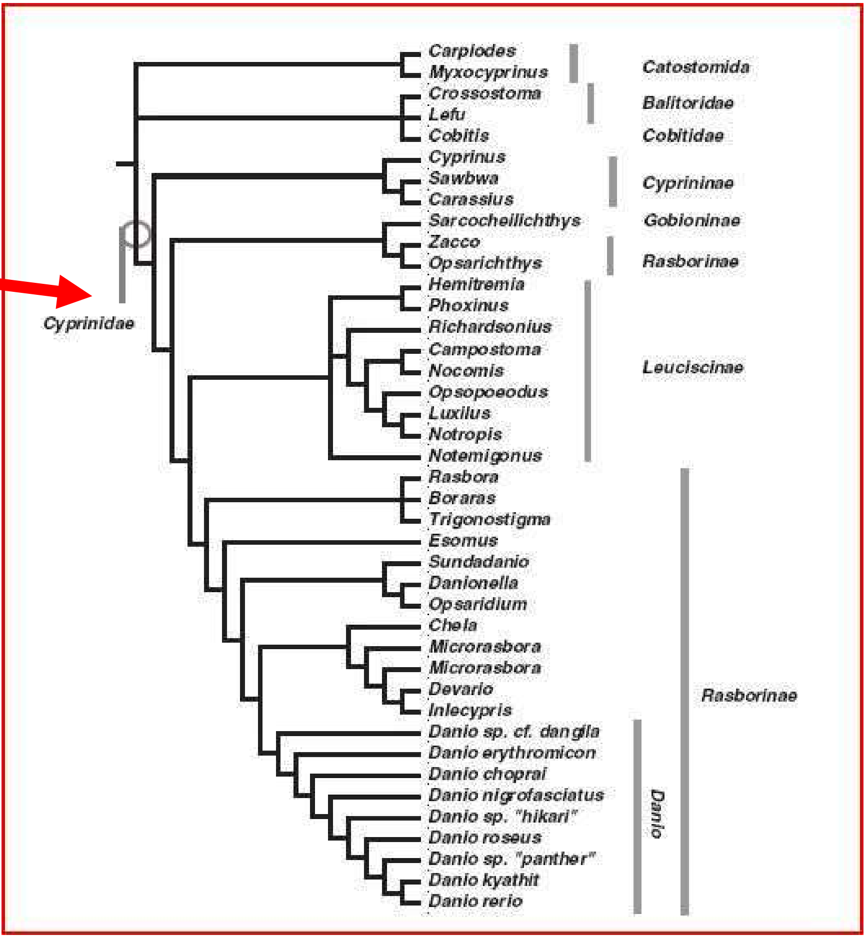


BOLDSYSTEMS

**BARCODE OF LIFE DATA SYSTEM**

http://www.boldsystems.org/

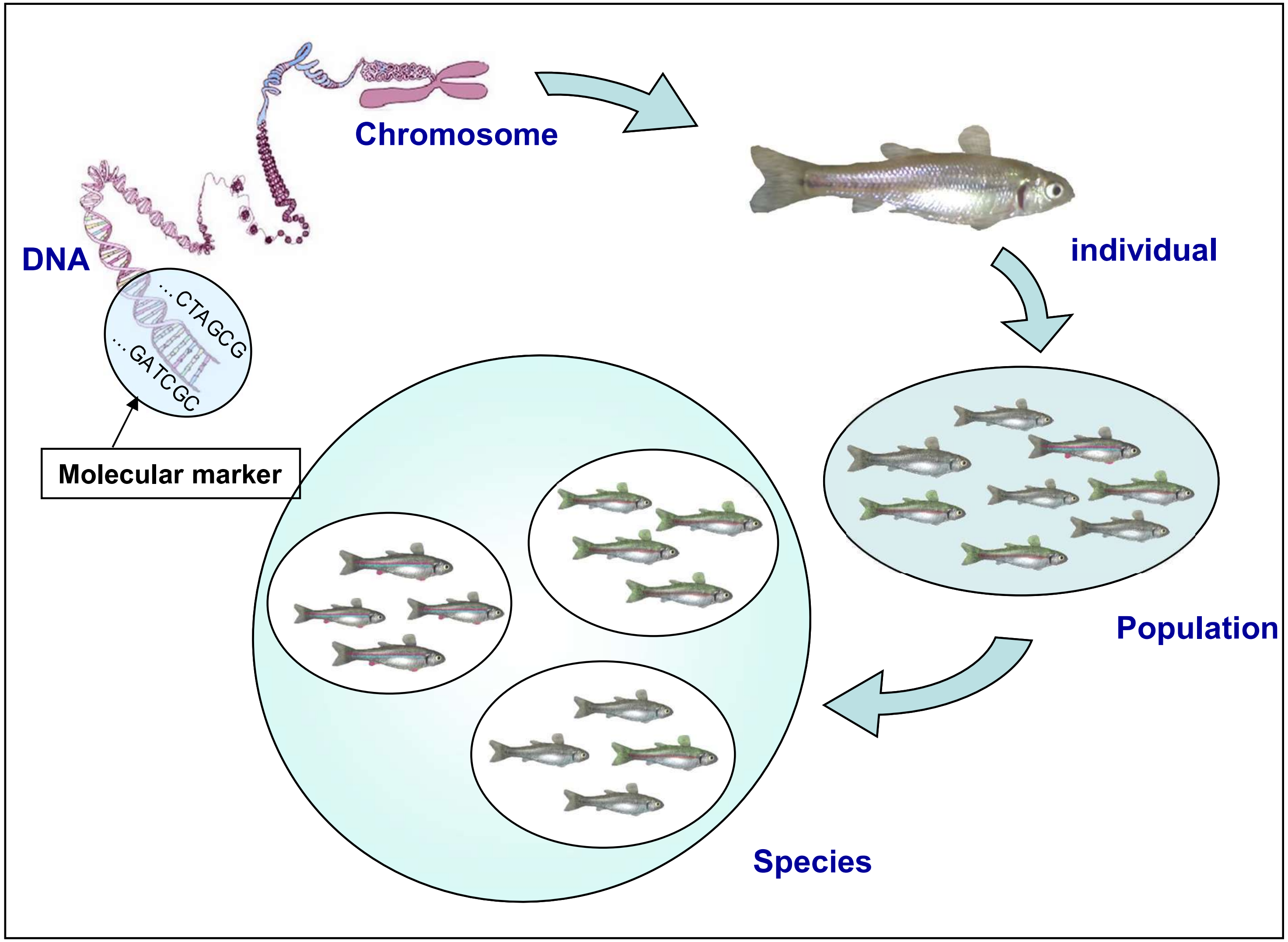
More than 22,607 fish species



Taxonomy

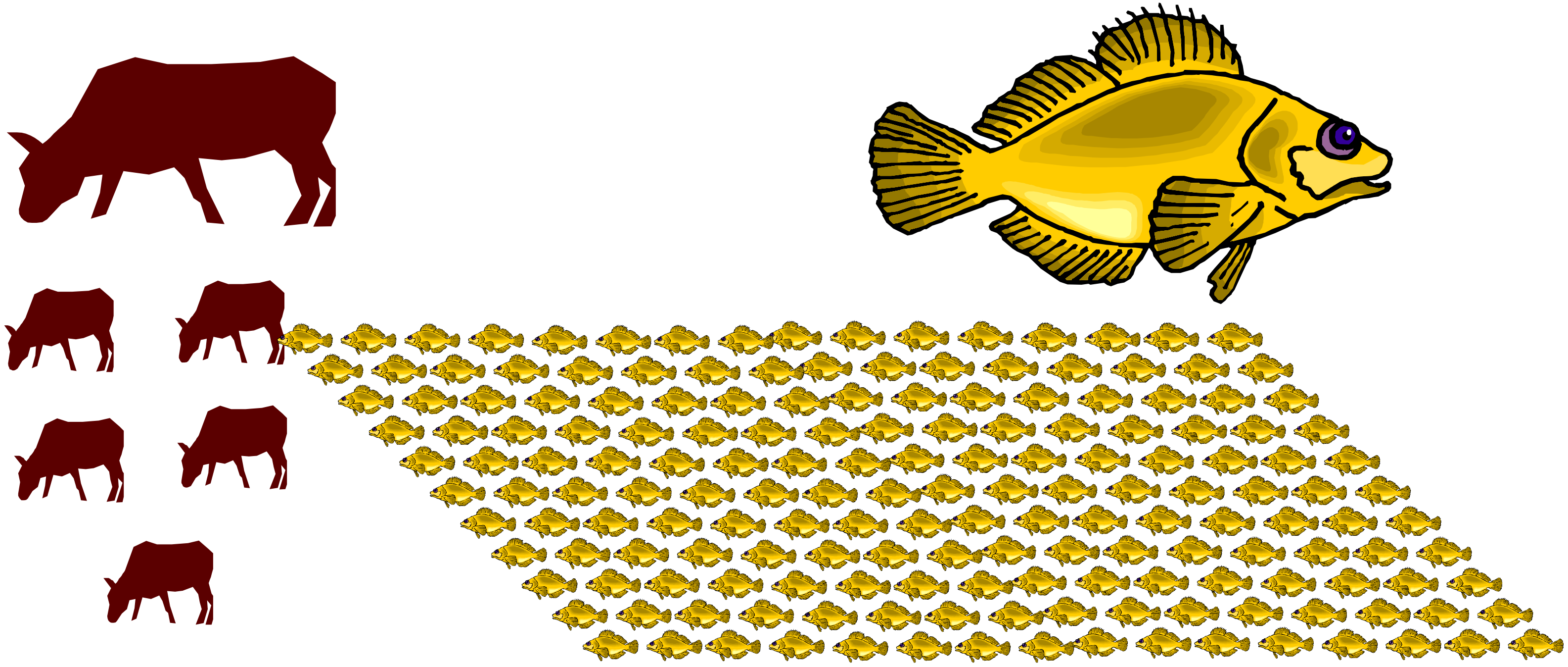
http://www.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=77

# Levels of the (population) genetics –different views



# Breeding characteristics of fish

- External fertilisation
- External development (strong environmental influence)
- High number of gametes
  - – Very High number of offspring



# Genetic definitions III

- **Gene** is a locatable region of genomic sequence, corresponding to a unit of inheritance, which is associated with regulatory regions, transcribed regions and/or other functional sequence regions

- An **allele** is one member of a pair or series of different forms of a gene.

- In biology, **mutations** are changes to the nucleotide sequence of the genetic material of an organism. Mutations can be caused by copying errors in the genetic material during cell division, by exposure to ultraviolet or ionizing radiation, chemical mutagens, or viruses, or can occur deliberately under cellular control during processes such as hypermutation.

# Genetic definitions II

- A **phenotype** is any *observable characteristic* of an organism, such as its morphology, development, biochemical or physiological properties, or behavior. Phenotypes result from the expression of an organism's genes as well as the influence of environmental factors and possible interactions between the two

- The **genotype** is the genetic constitution of a cell, an organism, or an individual (i.e. the specific allele makeup of the individual) usually with reference to a specific character under consideration

# Genetic definitions I

- In biology, a **species** is one of the basic units of biological classification and a taxonomic rank. A species is often defined as a group of organisms capable of interbreeding and producing fertile offspring. While in many cases this definition is adequate, more precise or differing measures are often used, such as based on similarity of DNA or morphology. Presence of specific locally adapted traits may further subdivide species into subspecies.

- **population** is the collection of inter-breeding organisms of a particular species

# Genetic definitions IV

- **Population genetics** is the study of the allele frequency distribution and change under the influence of the four evolutionary forces: selection, genetic drift, mutation and gene flow.

# Genetic examination of fish populations

Two reasons:

- Description the genetic variability of the population
- Forecast the changes in the population

Methods:

- Morphological examinations
- Molecular biology examination

# Qualitative characters

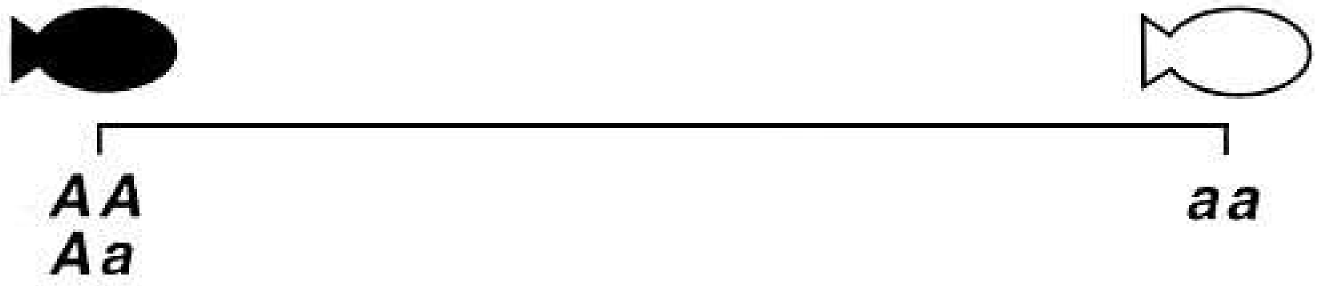
- Independent for effects of environment
- Manifestation in population is not continuous;
- They are determined one or oligo genes;
- Characters can not determined by unit of measurement/for example; kg,cm/
- Penetrance; 0-100 % /manifestation of recessive allel/--on *population* level
- expressivity; 0-100 % / manifestation of recessive allel/--on *individual* level

# Qualitative characters

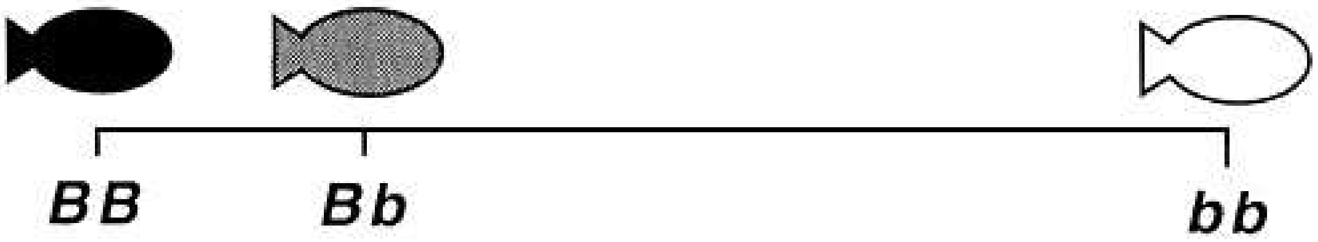
- Independent for effects of environment
- Manifestation in population is not continuous;
- They are determined one or few genes;
- Characters can not determined by unit of measurement/for example; kg,cm/
- Penetrance; 0-100 % /manifestation of recessive allel/--on *population* level
- expressivity; 0-100 % / manifestation of recessive allel/--on *individual* level

# Qualitative characters

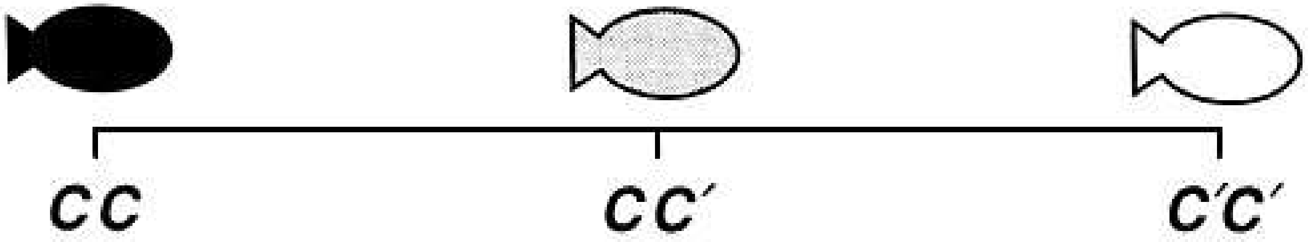
**Total dominancy**



**Partials dominancy**



**Intermedier inheritance**



# Dominant - recessive gene effect


© SOLENT NEWS AND PHOTOS

# Inheritance of Long Fins in Ornamental Koi Carp

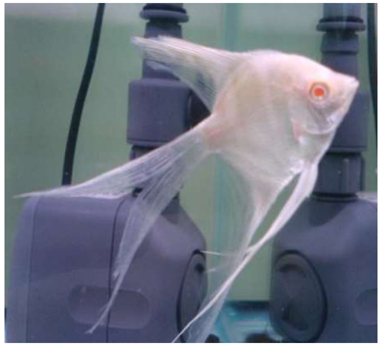
**Dominant mutation of one gene .**

***Fish with genotypes LfLf and Lflf have long fins,***

*North American Journal of Aquaculture 73:*

# Simple Dominancy

Homozygote a/a

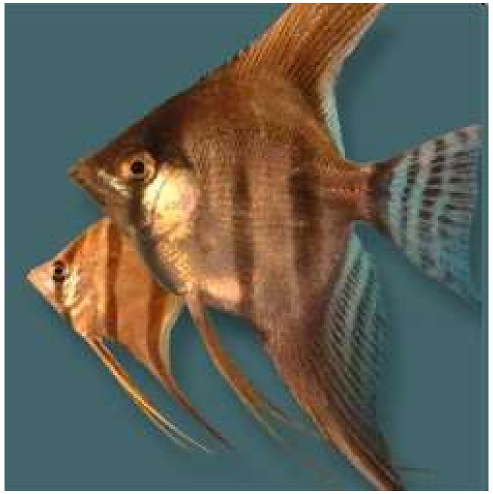


×

Homozygote A/A

→

Heterozygote A/a



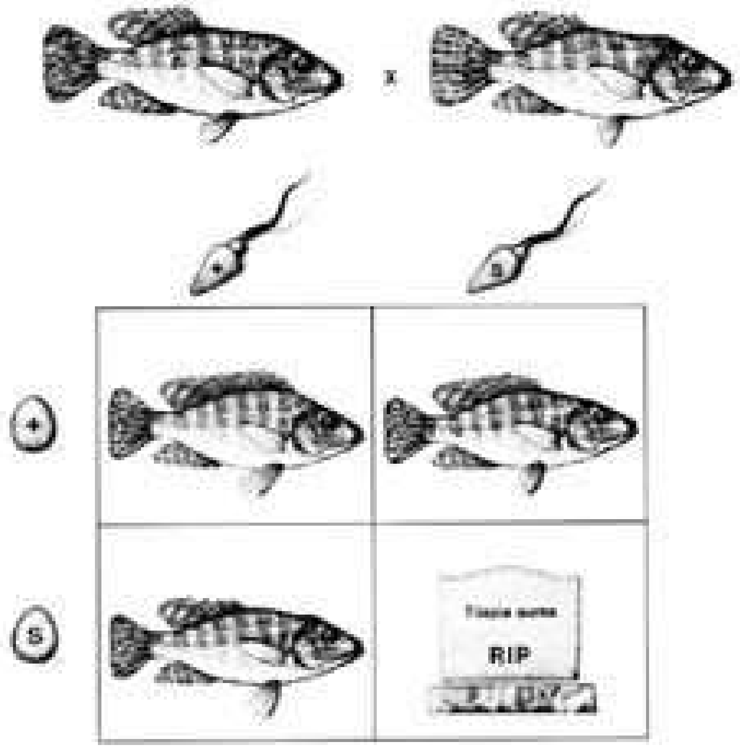
# Phenotypic markers


© Noel M. Burkhead

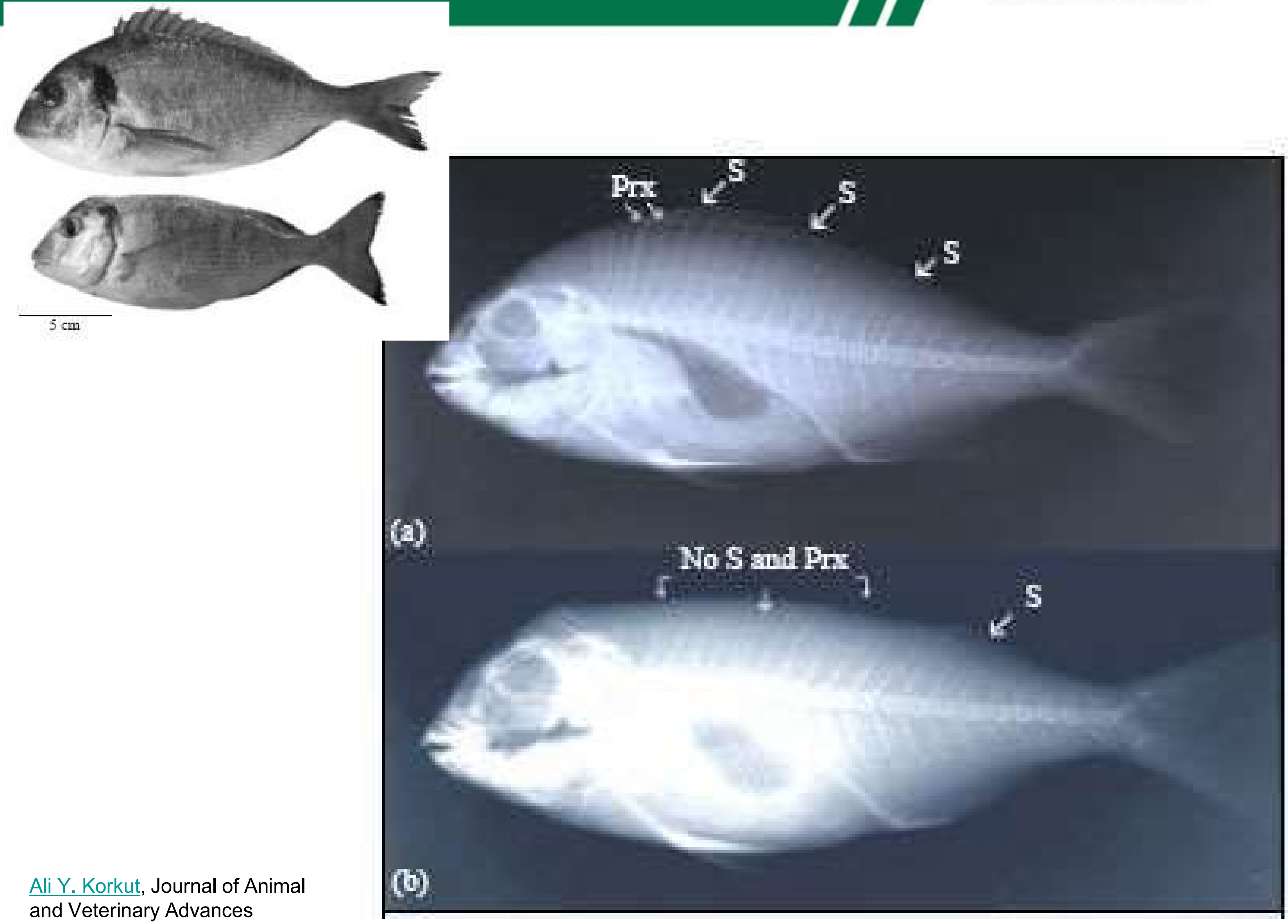
***Oreochromis aureus*-Saddleback**

**(**Tave *et al.* 2006; Auburn University , Israel)

- Dominant phenotype (Homozygous form is lethal)
- Heterozygous form
  - dorsal fin deformation
  - reduced survival rate (3 months 67%)
  - stress sensitivity



# Saddleback Syndrome in Cultured Gilthead Sea Bream (*Sparus aurata* L.)



Ali Y. Korkut, Journal of Animal and Veterinary Advances 2009 ;8 : 11 ; 2360-2362

# Intermediet inheritance


Homozygote B/B


Homozygote b/b


Heterozygote B/b

# Itra-allelic inheritance

<u>Lethal genes:</u>

Xiphophorus helleri - Simpson tail

<u>Semilethal:</u>

Xiphophorus helleri - Líra tail

Ictalurus punctatus - albinisms

Xiphophorus helleri - Berliner spots

# Intra allelic inherritance

palomino trout (golden rainbow trout x rainbow trout)

# Pleiotropic effect

Rainbow trout– blue skin color– more varieties

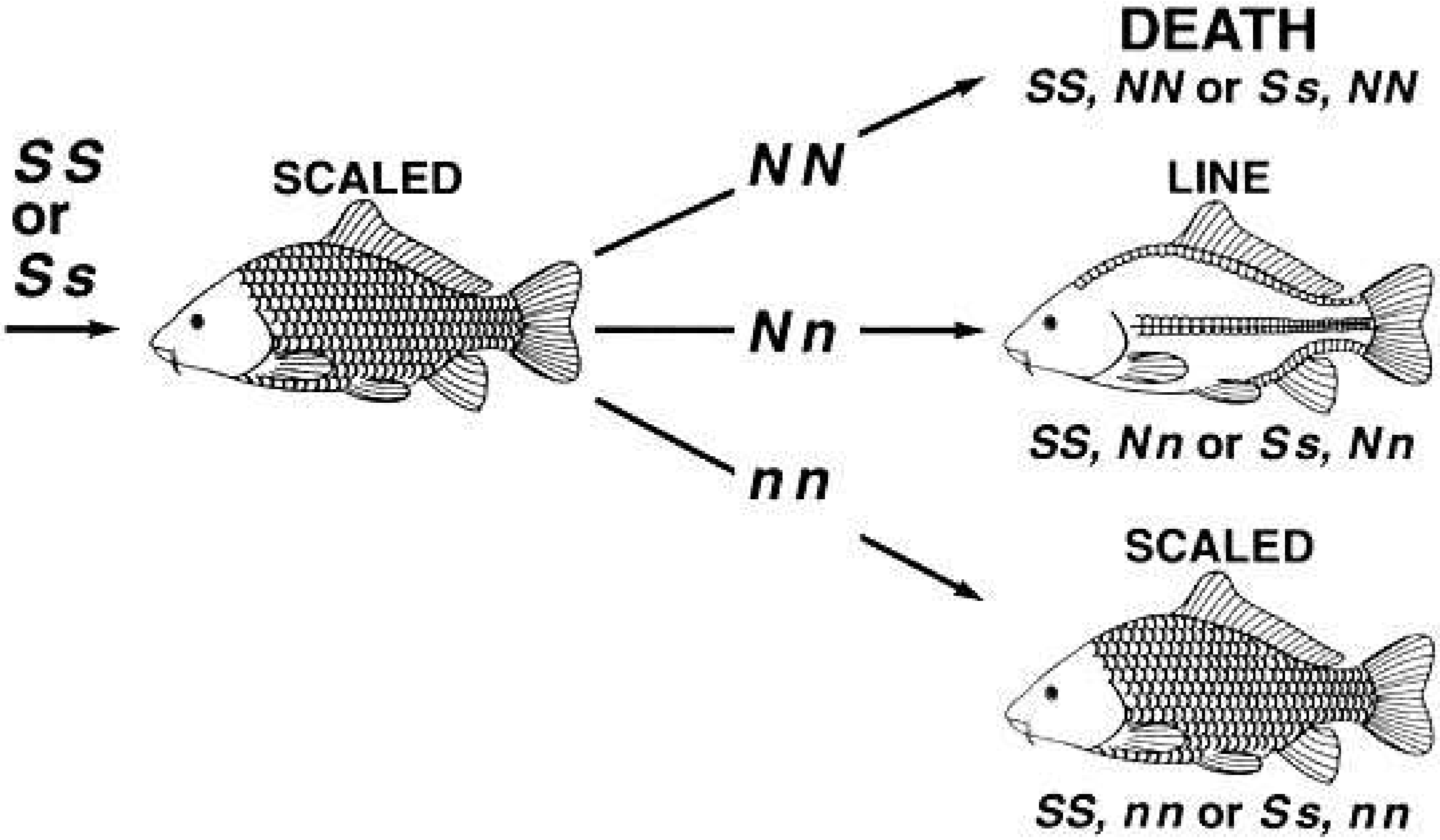
Yamazaki 1974- Japan (cobalt blue)
- Obesity
- liver and kidney degeneration

Kincaid 1975- Great Britain (iridescent metallic blue)
- Increased growth rate

Blanc, 2006- France (cobalt blue)
- 25% production deficit
- Lower survival rate
- Smaller body size

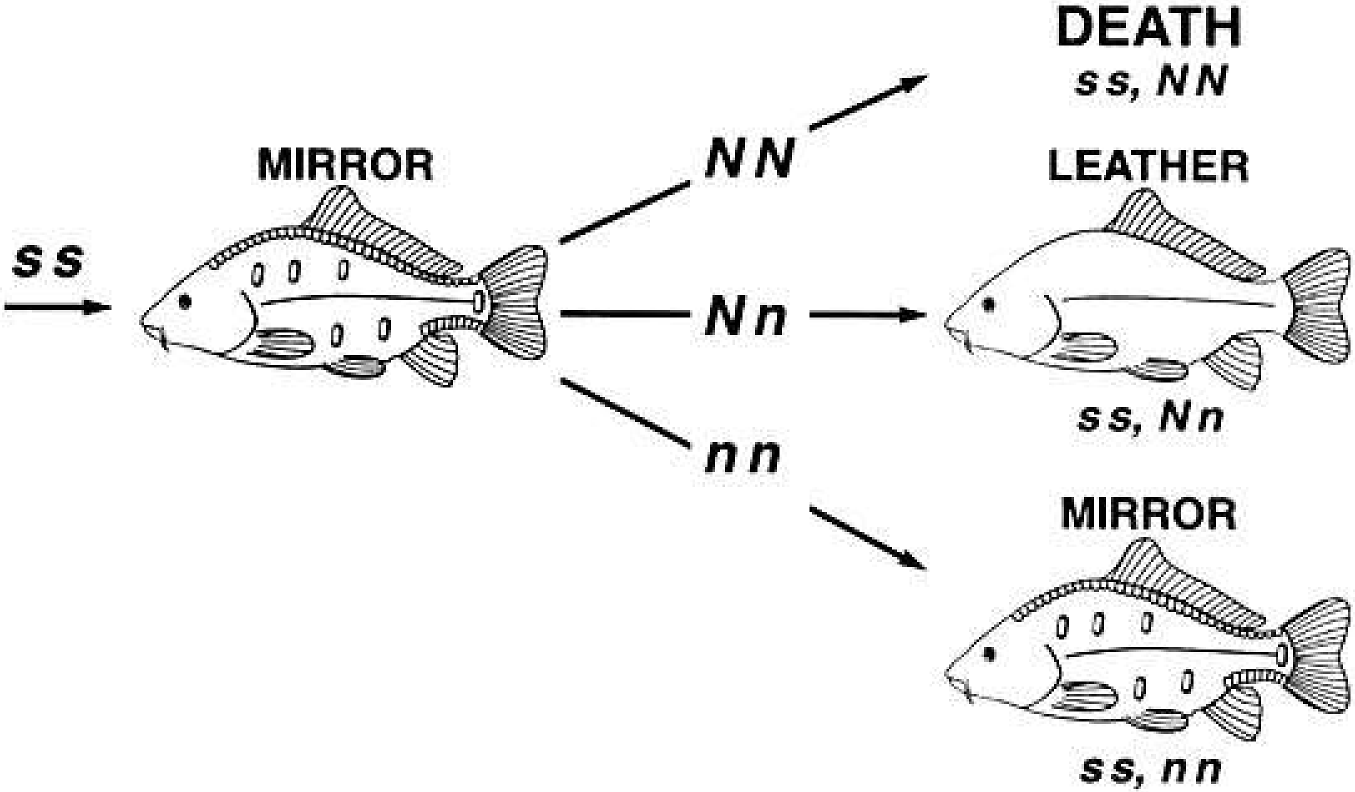
# Cumulateive, Epistaticc, Pleiotropic gene effekt

Scale patter in carp 1



# Cumulateive, Epistaticc, Pleiotropic gene effekt

Scale patter in carp 2



# Color inheritance1.

鯉

Koi/Showa History?

1850 – 1870 ??

Tetsu Magoi
Iron Carp

+

Red Carp
Hi Goi

=

Aka & Ki Bekko

ry?

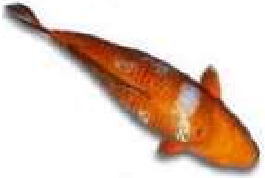
eparation”

ドイツ
Doitsu

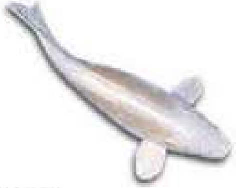
べっ甲
Bekko

浅黄
Asagi

銀紅白
GinRin Kohaku



Kawarimono

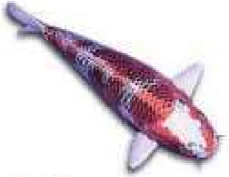


Hikari

五色
Goshiki

紅白
Kohaku

九紋龍
kumonryu

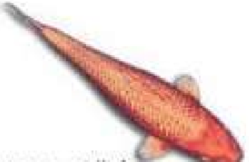


Kujaku

菊衣
Goromo

銀松葉
Gin Matsuba

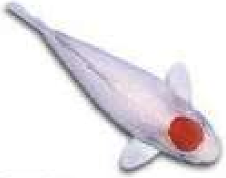
Sanke



オレンジ黄金
Orenji Ogon

プラチナ黄金
Platinum Ogon

落葉
Ochiba



Tancho

Tancho Sanke

昭和
Showa

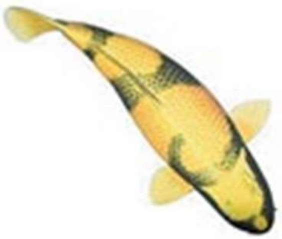
秋翠
Shusui

Utsuri

写り

Yamabuki Ogon

山吹黄金



Kin Ki Utsuri

Kin Hi Utsuri

Kin Showa

# Quantitative characters

- They are dependent by environment /different characters in different rate/
- They are describable by unit of measurement, for example: weight in kg or length in mm
- They show variety around the arithmetic mean /Gauss-curve /
- They inherit as polygenes, there are complicated interaction among these genes, and not describable by Mendel's laws
- Their manifestations are continuous, and characterizable by  variances and these variances increase gradually from generation to generation.

# Variance Components of a Quantitative Trait / Phenotype

- **VP = VG + VE + VGE**
- VP = total phenotypic variation of the segregating population
- VG = genetic variation that contributes to the total phenotypic variation
- VE = environmental contribution to the total phenotypic variation
- VGE = variation associated with the genetic and environmental factor interactions

# Variance Components of a genetic variance

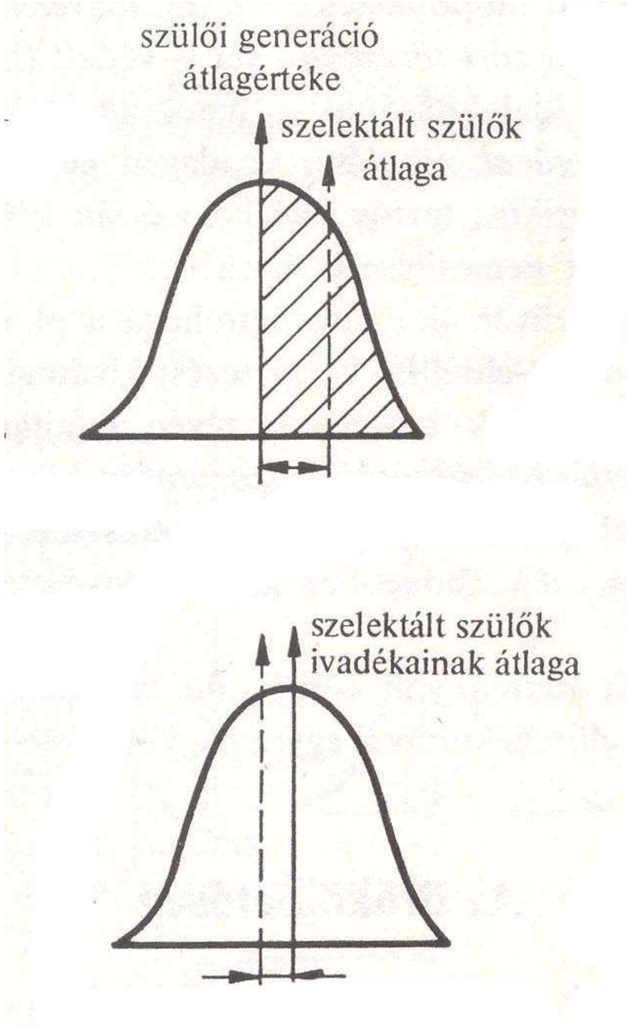
- **VG = VA + VD + VI**
- The **additive genetic variance (VA)**. Genetic variance associated with the average effects of substituting one allele for another.
-
- The **dominance genetic variance (VD)**. Genetic variance at a single locus attributable to dominance of one allele over another
- The **interaction genetic variance (VI)**. The genetic basis of this variance is epistasis. (The epistasis is the interaction between genes.)

# The heritability

- In genetics, **Heritability** is the proportion of phenotypic variation in a population that is attributable to genetic variation among individuals.

$$h^2 = \frac{R}{S}$$

S = selection Differential
R= Selection drift (GAIN)



F1

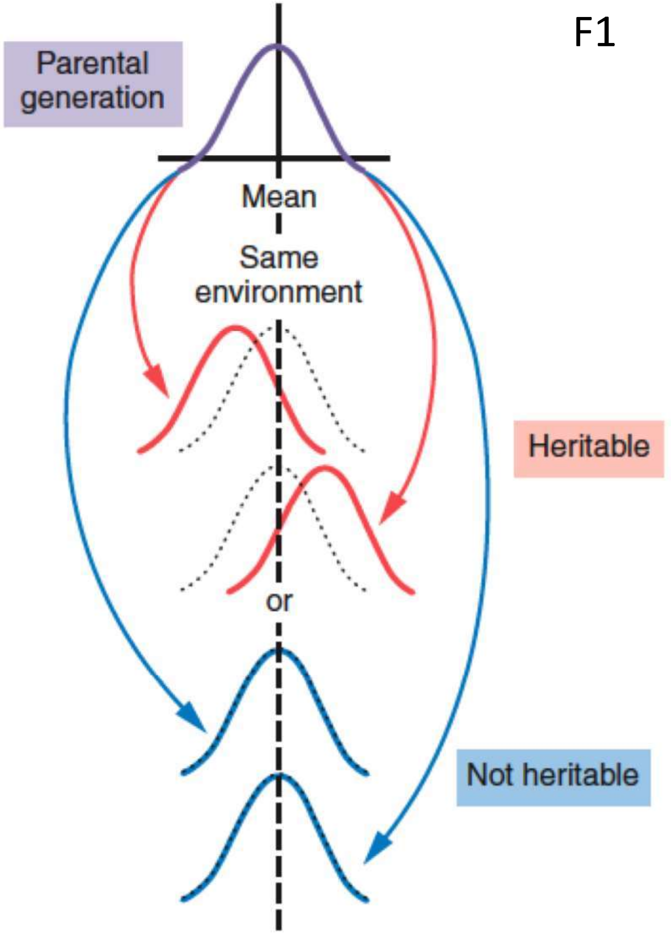
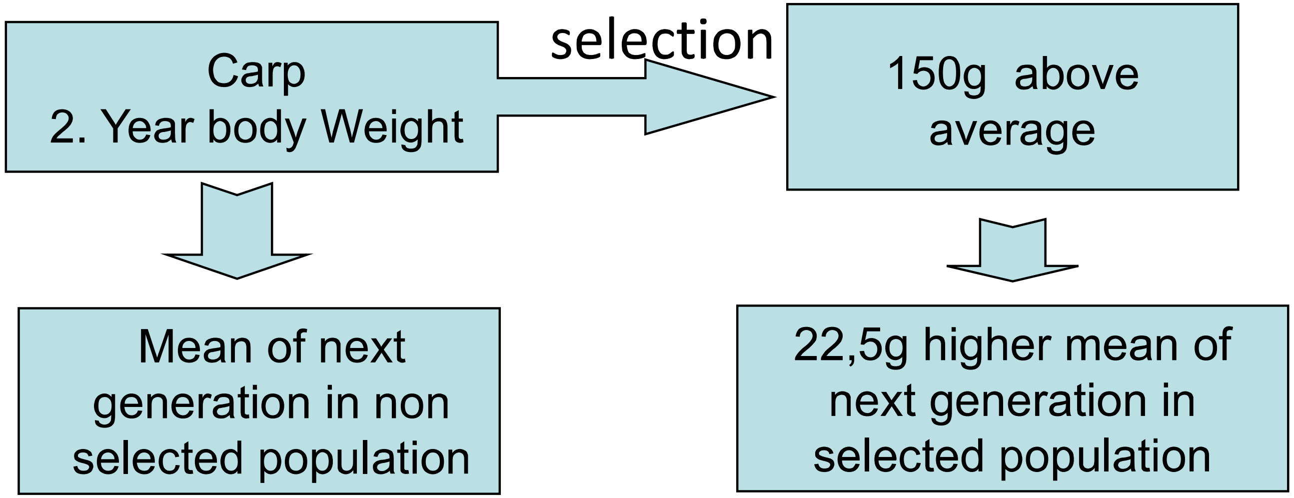


**Figure 20-10 Standard method for testing for heritability in experimental organisms.** Crosses are performed within two populations of individuals selected from the extremes of the phenotypic distribution in the parental generation. If the phenotypic distributions of the two groups of offspring are significantly different from each other (red curves), then the character difference is heritable. If both offspring distributions resemble the distribution for the parental generation (blue curves), then the phenotypic difference is not heritable.

# The heritability



Carp
2. Year body Weight

selection

150g above average

Mean of next generation in non selected population

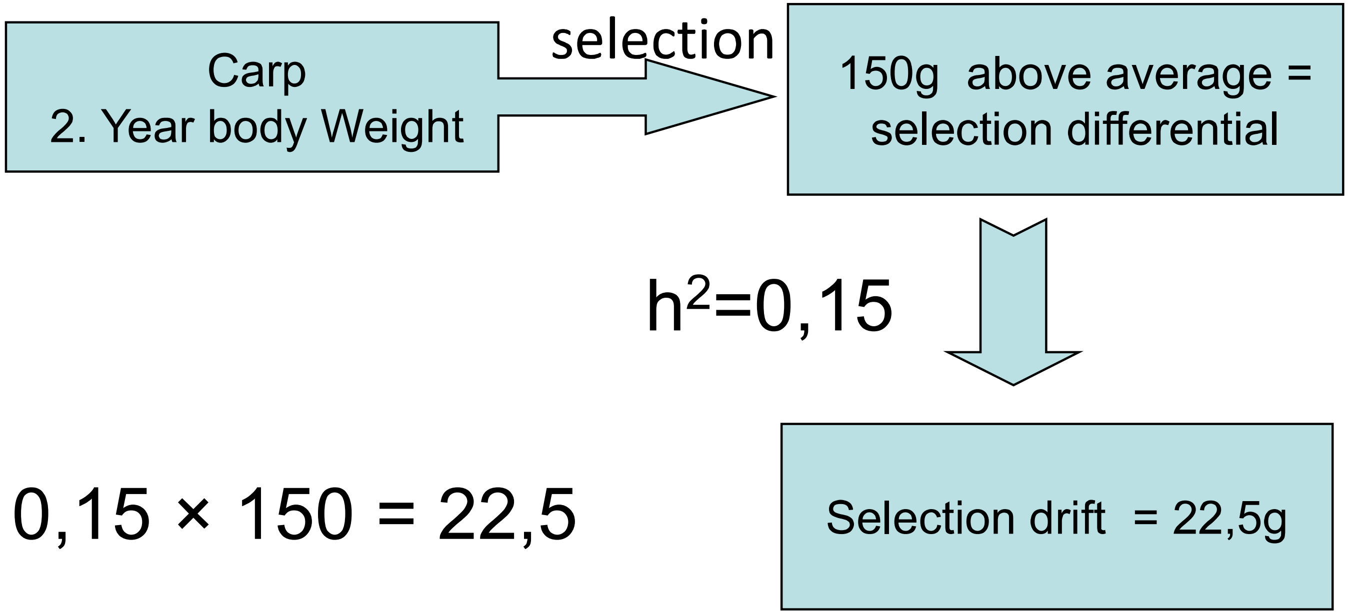
22,5g higher mean of next generation in selected population

$$h^2 = \frac{22,5}{150} = 0,15$$

# The heritability

$$h^2 = \frac{R}{S} \longrightarrow R = h^2 \times S$$

Selection drift can be predicted with known heritability and selection differential



Carp
2. Year body Weight

selection

150g above average = selection differential

$h^2 = 0{,}15$

$0{,}15 \times 150 = 22{,}5$

Selection drift = 22,5g

# Applications of $h^2$

- choose of method of selection
  - high $h^2$ ⇒ phenotypical selection
  - low $h^2$ ⇒ family selection
- choose of method of breeding
  - high ⇒ single cross with excellent parents
  - low ⇒ combination cross, intraspecific cross
- estimate of efficiency of selection
  - high ⇒ better
  - slow ⇒ worse
- Choose which characters will be selected and how.
  - high ⇒ better
  - slow ⇒ worse

# $h^2$ in fish

- carp according to Kirpichnyikov :

body weight: 0.1-0.2
body length: 0.2-0.3
head length: 0.4-0.5
back height: 0.45-0.55
back widht:  0.3-0.35

| | | |
|---|---|---|
| Nile tilapia | 4-week weight | 0.06 |
| | 10-week weight | 0.46 |
| | 7-month weight (realized) | 0.05 |
| | 45-day length | 0.10 |
| | 90-day length | 0.06 |
| | fecundity at first spawning | 0.09 |
| Mozambique tilapia | 5-month weight, female (realized) | 0.01 |
| | 5-month weight, male (realized) | 0.10 |

# $h^2$ in fish

- **1. Phenotypic features:** (h2=0.0-0.4)

eg. scalyness or color

- **2. Etology, behaviour features:** (h2=0.0-1.0)

eg. time and place of spawning,

- **3. Morphological characters** (h2=0.1-0.4)

eg. body weight, body lenght, head lenght

description: profilindex (muscles system and types of carp), headindex (feeding factors),

- **4. Meristic features** (h2=0.4-0.7)

number of: scales on the lateral line, rays in different fins, teeth of pharyngal etc.

# $h^2$ in fish

- **5. Biochemical markers** (h2= 1 kodominantly inheritance)

proteins (enzymes) eg. Lactate dehydrogenase (LDH) or Transferrine (Tf)

- **6. Immune genetic markers** (h2= 1)

Major and minor histocompatibility genes (app. 30 genes with many alleles)

- **7. DNA markers** (h2= 1)

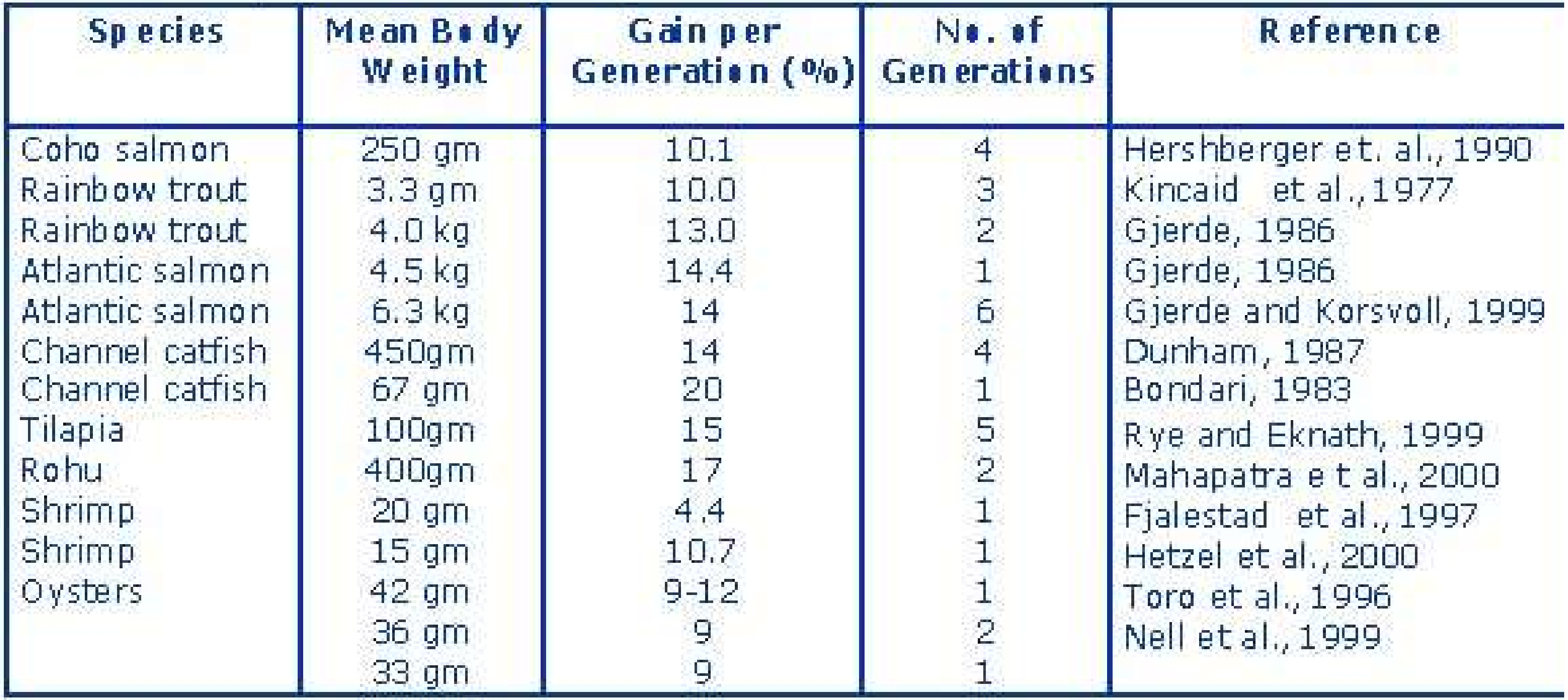
from: chromosomes, nuclear DNA, mithochodrial DNA

methods: **chromosome analysis**, **RFLP** (**R**estriction **F**ragment **L**ength **P**olymorphisms, DNA fingerprinting), **RAPD, PCR** (**P**olymerase **C**hain **R**eaction), **VNTR** (**V**ariable **N**umbers of **T**andem **R**epeats microsatellits).

# Correlation within traits

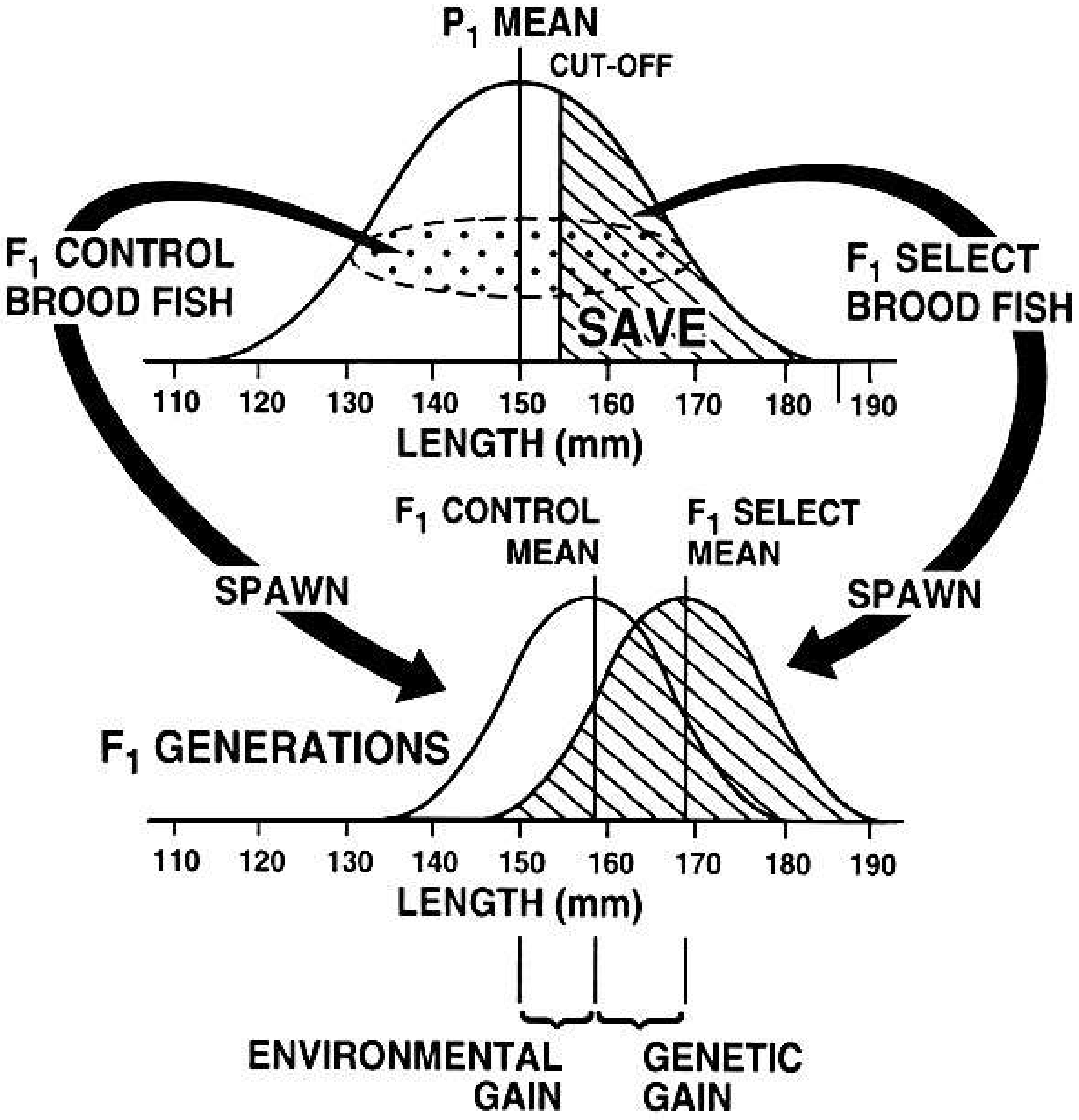
| Tulajdonság I | Tulajdonság II | Korrelációs érték |
|---|---|---|
| növekedés | tápa. hasznosítás | 0,99 |
| növekedés | hús/zsír arány | -0,47 |
| növekedés | gonádsúly | -0,49 |
| növekedés | termékenység | -0,59 |
| hús/zsír arány | tápa. hasznosítás | -0,40 |
| hús/zsír arány | gonádsúly | -0,29 |

**Table 2.** Response to selection in growth rate



| Species | Mean Body Weight | Gain per Generation (%) | No. of Generations | Reference |
|---|---|---|---|---|
| Coho salmon | 250 gm | 10.1 | 4 | Hershberger et. al., 1990 |
| Rainbow trout | 3.3 gm | 10.0 | 3 | Kincaid et al., 1977 |
| Rainbow trout | 4.0 kg | 13.0 | 2 | Gjerde, 1986 |
| Atlantic salmon | 4.5 kg | 14.4 | 1 | Gjerde, 1986 |
| Atlantic salmon | 6.3 kg | 14 | 6 | Gjerde and Korsvoll, 1999 |
| Channel catfish | 450gm | 14 | 4 | Dunham, 1987 |
| Channel catfish | 67 gm | 20 | 1 | Bondari, 1983 |
| Tilapia | 100gm | 15 | 5 | Rye and Eknath, 1999 |
| Rohu | 400gm | 17 | 2 | Mahapatra et al., 2000 |
| Shrimp | 20 gm | 4.4 | 1 | Fjalestad et al., 1997 |
| Shrimp | 15 gm | 10.7 | 1 | Hetzel et al., 2000 |
| Oysters | 42 gm | 9-12 | 1 | Toro et al., 1996 |
|  | 36 gm | 9 | 2 | Nell et al., 1999 |
|  | 33 gm | 9 | 1 |  |

# Control population is always required



# Breeding 1.

Breeding: continuous controlled crossing of selected specimens for better offspring

Method influenced by:

- species
- generation time (influences by natural conditions at fish)
  - puberty
  - breeding maturity
- Level of the breeding (species , subspecies, variety , landraces)
- aims of breeding
- breeding system

# Breeding 2.

Possible aims:

- Weight gain
- feed conversion
- reproducibility
- survival rate
- Resistance
- meat Quality

Complex aims:

- preservation traits + development of a trait

- development of more traits

- preservation some traits + development of more traits

# Breeding 3.

Breeding methods:

- Selection is made on the basis of selection criteria, limits (qualitative-quantitative traits, heritability h2, etc.).

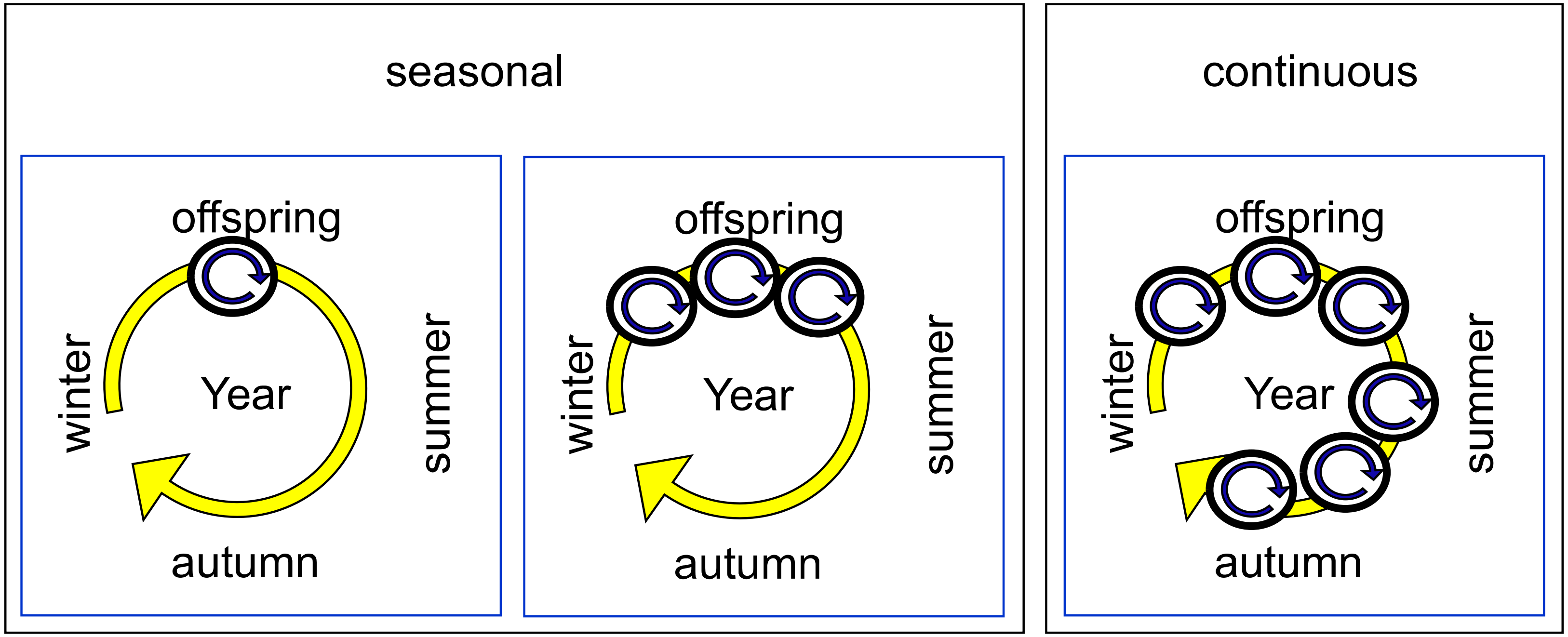
  - Simple selection
    - phenotypic selection
    - alternating selection
    - more independent selection criteria/borders

  - selection index
    - Dependent selection on the basis of calculation

  - family selection

# Breeding 4.

Breeding system:



Origin of breeders:

- producing stocks
- selected stocks

# New landrace

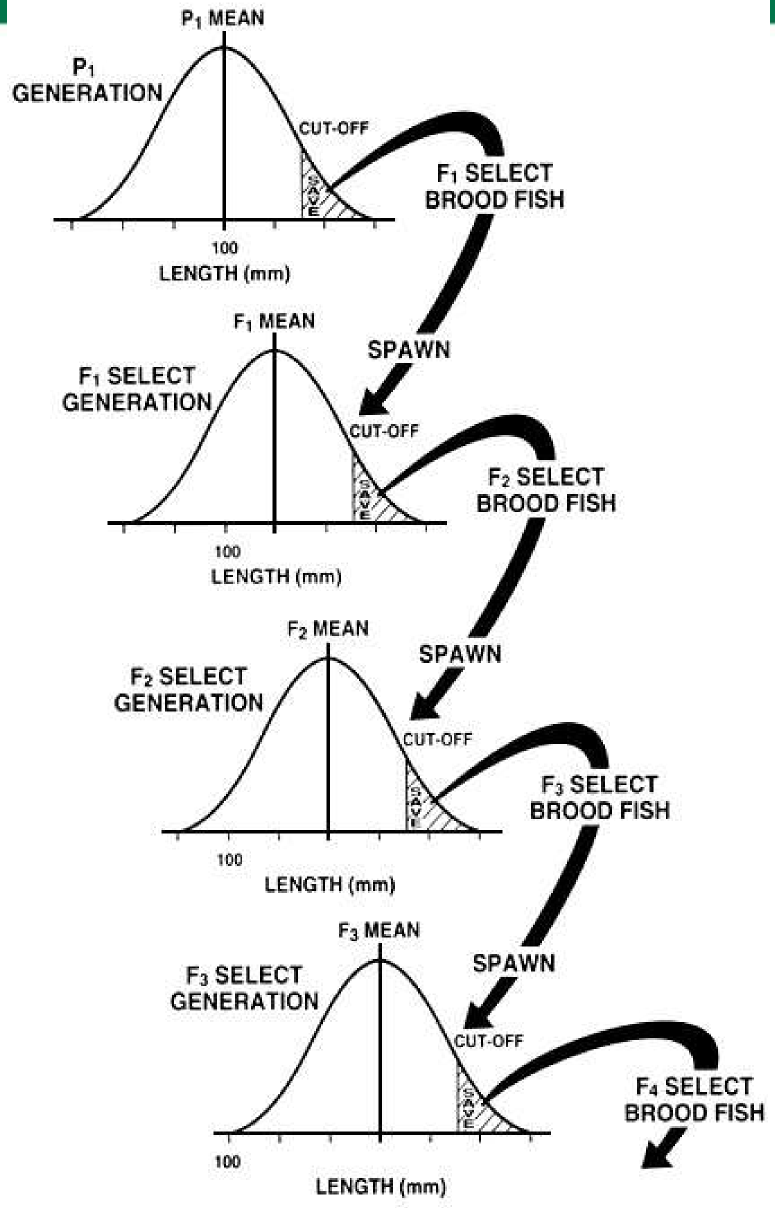
uniform phenotype,
Typical characteristics
appropriate production parameters

Breeding population

Suitable methods to reduce (genetic) diversity:

- selection
- inbreeding

# Simple selection



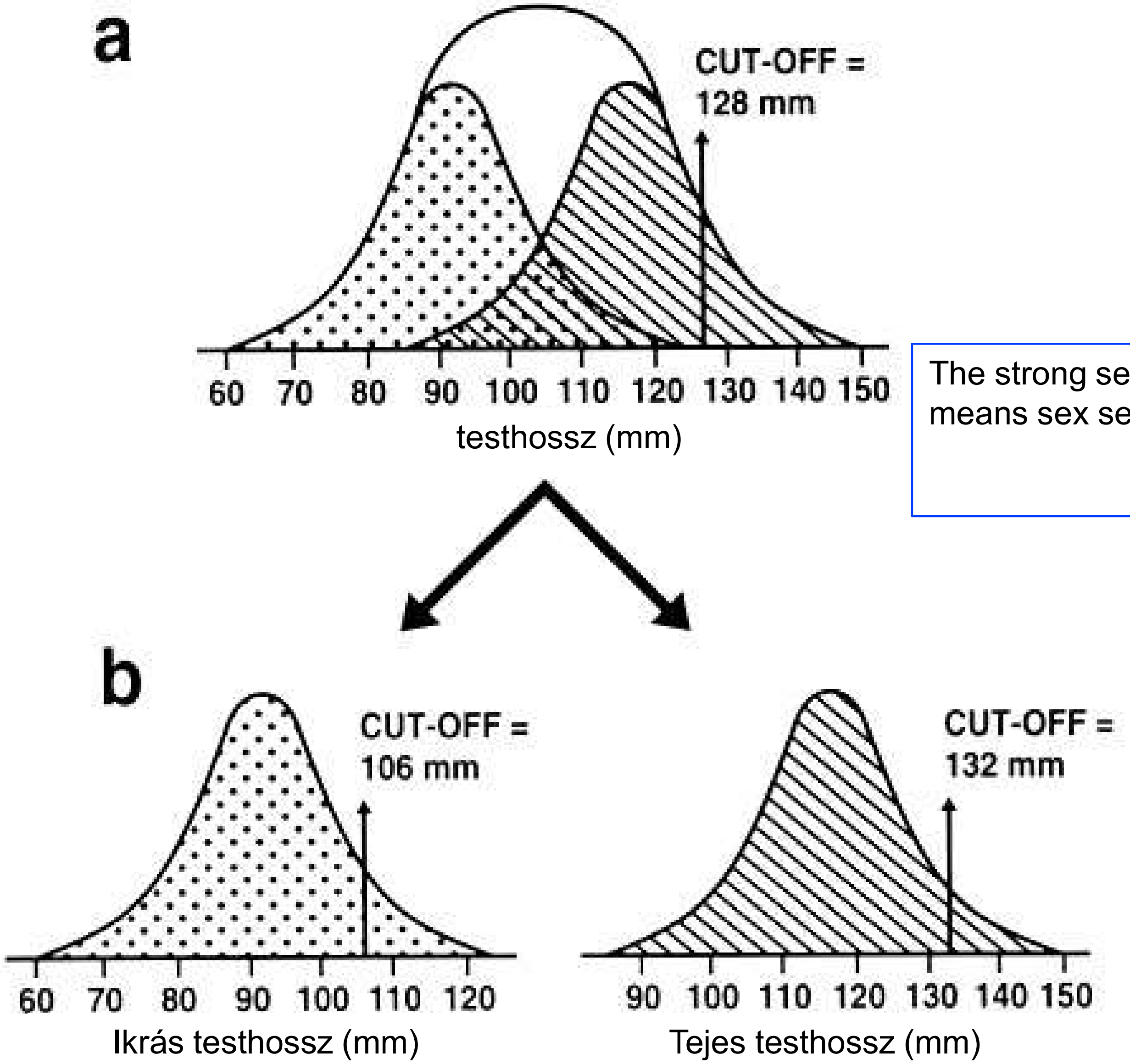
- Generation to generation ( don't mix the generations)

- Don't use breeders from outside the stock

**Table 2.** Response to selection in growth rate

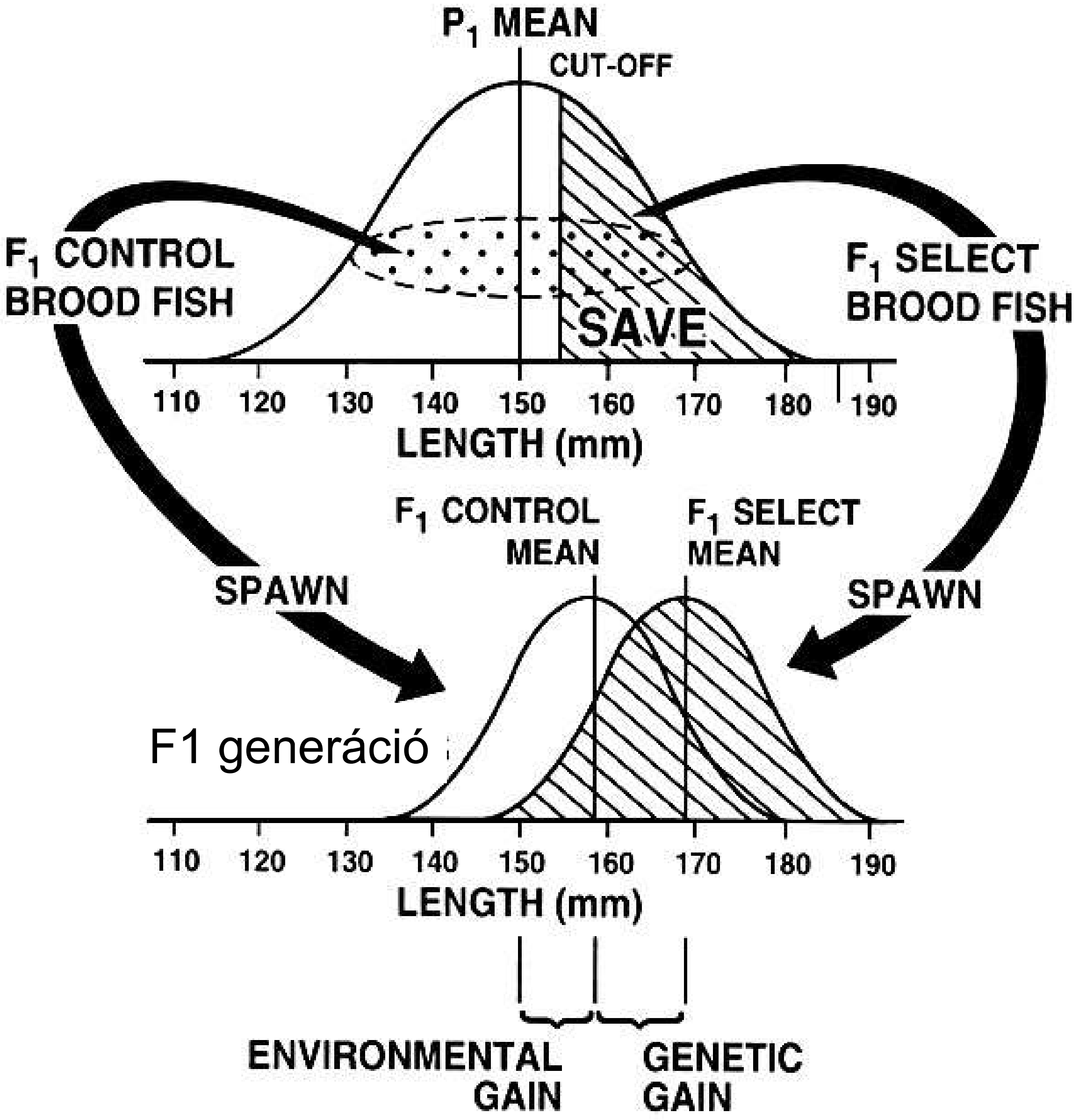
| Species | Mean Body Weight | Gain per Generation (%) | No. of Generations | Reference |
|---|---|---|---|---|
| Coho salmon | 250 gm | 10.1 | 4 | Hershberger et. al., 1990 |
| Rainbow trout | 3.3 gm | 10.0 | 3 | Kincaid et al., 1977 |
| Rainbow trout | 4.0 kg | 13.0 | 2 | Gjerde, 1986 |
| Atlantic salmon | 4.5 kg | 14.4 | 1 | Gjerde, 1986 |
| Atlantic salmon | 6.3 kg | 14 | 6 | Gjerde and Korsvoll, 1999 |
| Channel catfish | 450gm | 14 | 4 | Dunham, 1987 |
| Channel catfish | 67 gm | 20 | 1 | Bondari, 1983 |
| Tilapia | 100gm | 15 | 5 | Rye and Eknath, 1999 |
| Rohu | 400gm | 17 | 2 | Mahapatra et al., 2000 |
| Shrimp | 20 gm | 4.4 | 1 | Fjalestad et al., 1997 |
| Shrimp | 15 gm | 10.7 | 1 | Hetzel et al., 2000 |
| Oysters | 42 gm | 9-12 | 1 | Toro et al., 1996 |
|  | 36 gm | 9 | 2 | Nell et al., 1999 |
|  | 33 gm | 9 | 1 |  |

# sexual dimorphism



The strong selection criteria means sex selection too

# Controll population



# Inbreeding 1.

***The aim:***

Increasing the homozygosity.
Preservation of required traits.

implementation:
Close inbreeding of close relatives : backcross, half-sib, full-sib crosses.

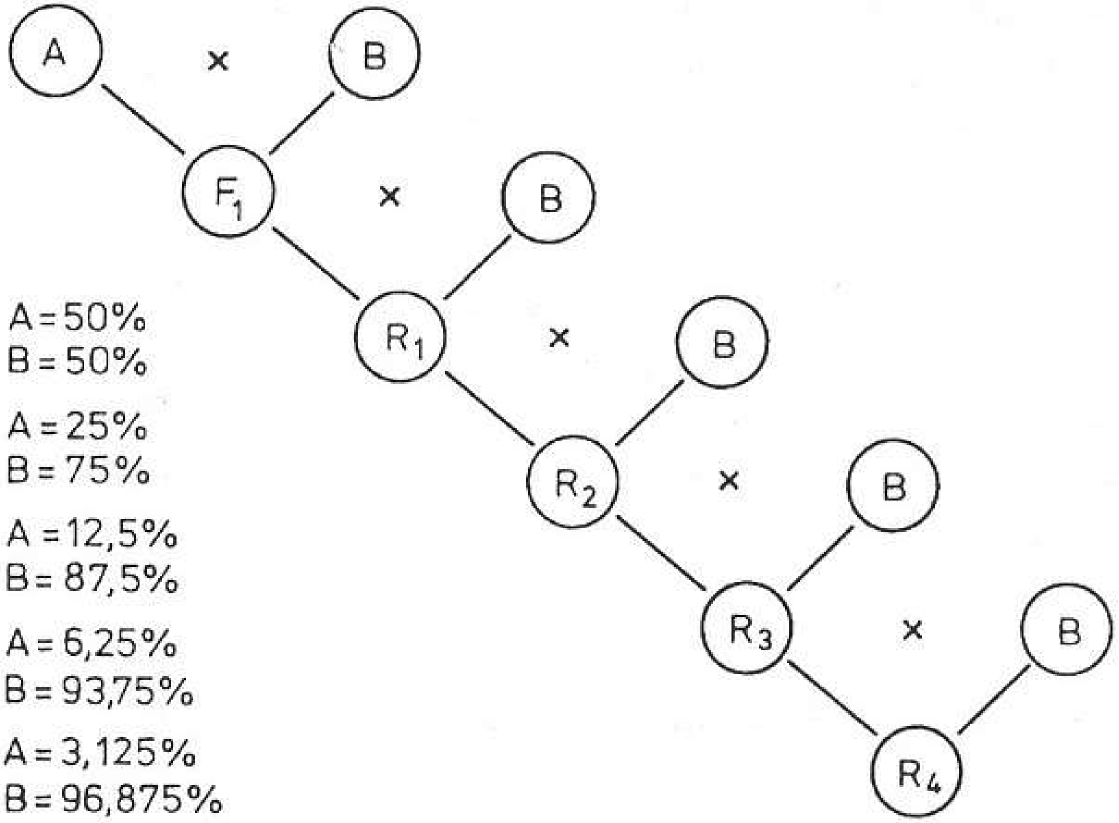
As a fact of inbreeding reduced the growth rate, reproductively, vitality, and adaptability can be observed..

*full-sib cross*

| | | |
|---|---|---|
| $P_0$ | ♀ X ♂ | 100% |
| $F_1$ | ♀ X ♂ | 50% |
| $F_2$ | ♀ X ♂ | 75% |
| $F_3$ | ♀ X ♂ | 87,5% |
| $F_4$ | ♀ X ♂ | 93,75% |
| $F_5$ | ♀ X ♂ | 96,875% |
| $F_6$ | ♀ X ♂ | 98,4375% |

# Crosses in the practice 1.

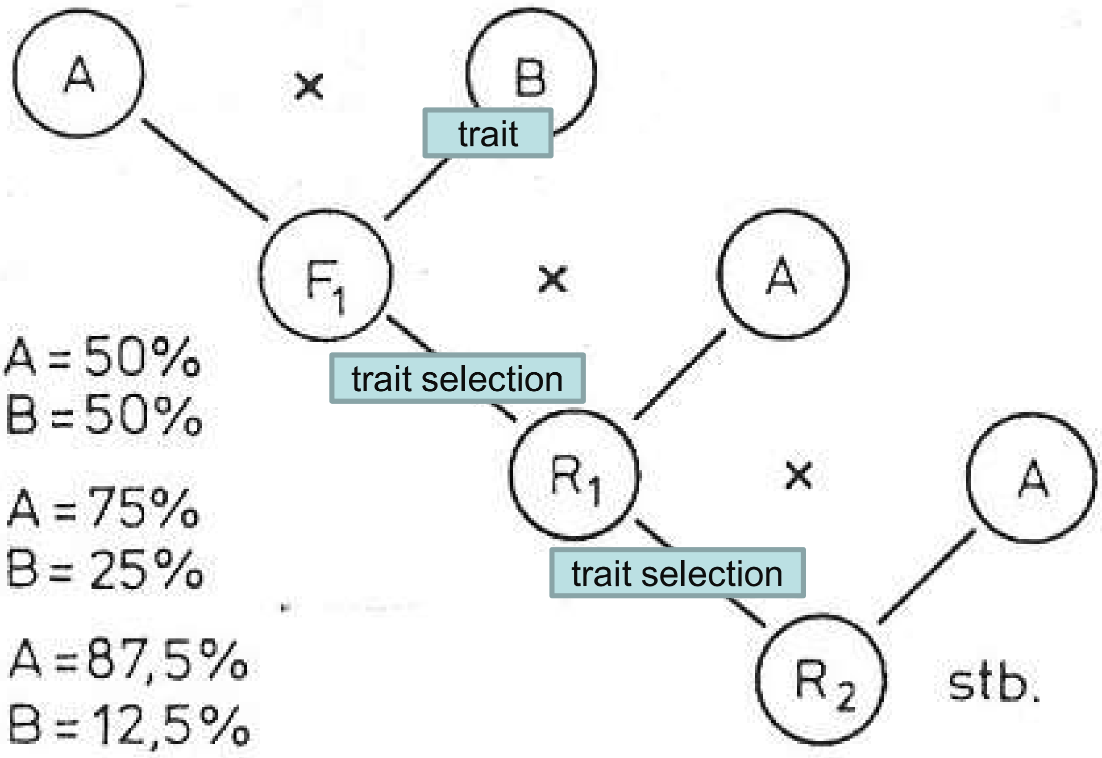
Breed (A) transformation to another (B) by continuous crossing .



A kiinduló fajta génaránya nemzedékenként feleződik.
B átalakitó fajta génaránya nemzedékenként nő.

# Crosses in the practice 2.

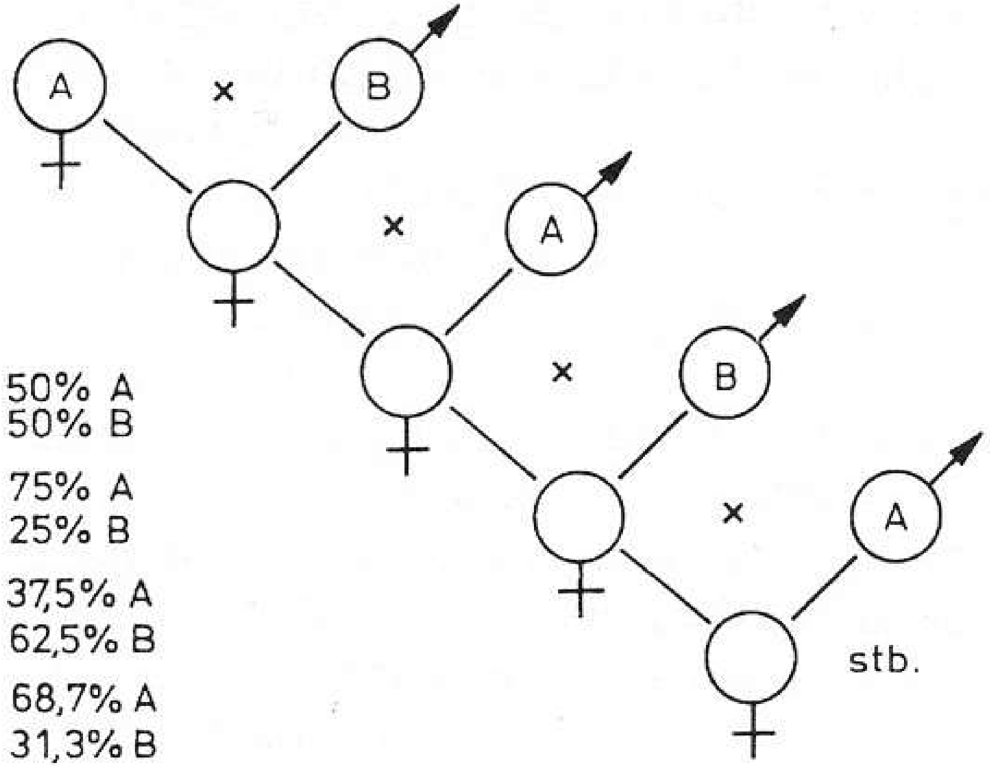
Improving of breed A with a trait of variety B.



Selection for the trait

# Direct livestock production by cross-breeding 1.

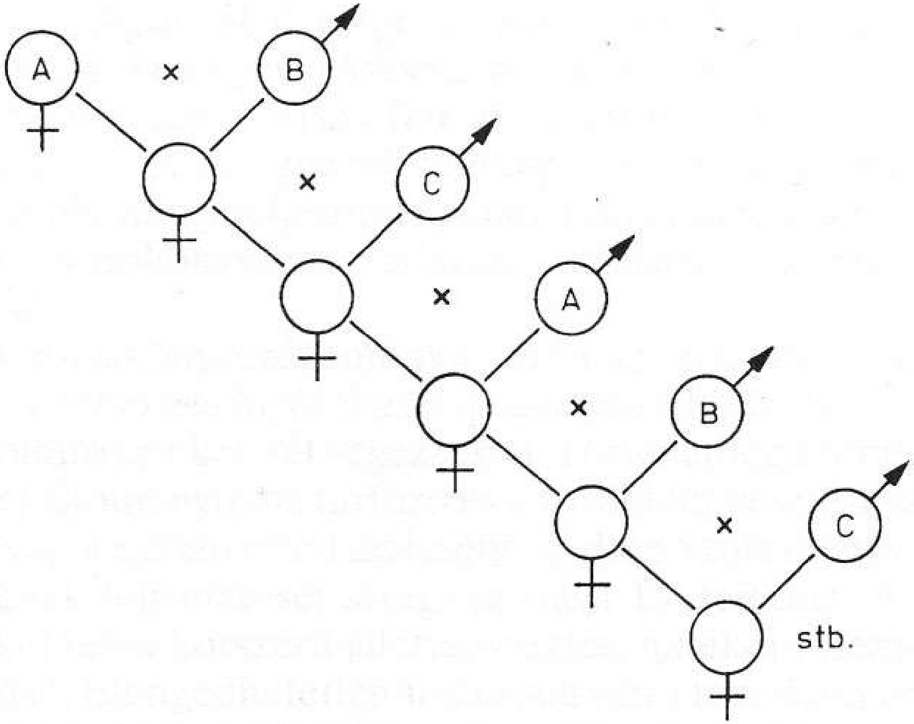
Rotation cross with two breeds



The method continuously maintaining the heterosis effect. However, heterozygosity will decline from the F2 generation. (After 6 generations, ~ 66% -33% alternates)

# Direct livestock production by cross-breeding 2.

Rotation cross with three breeds

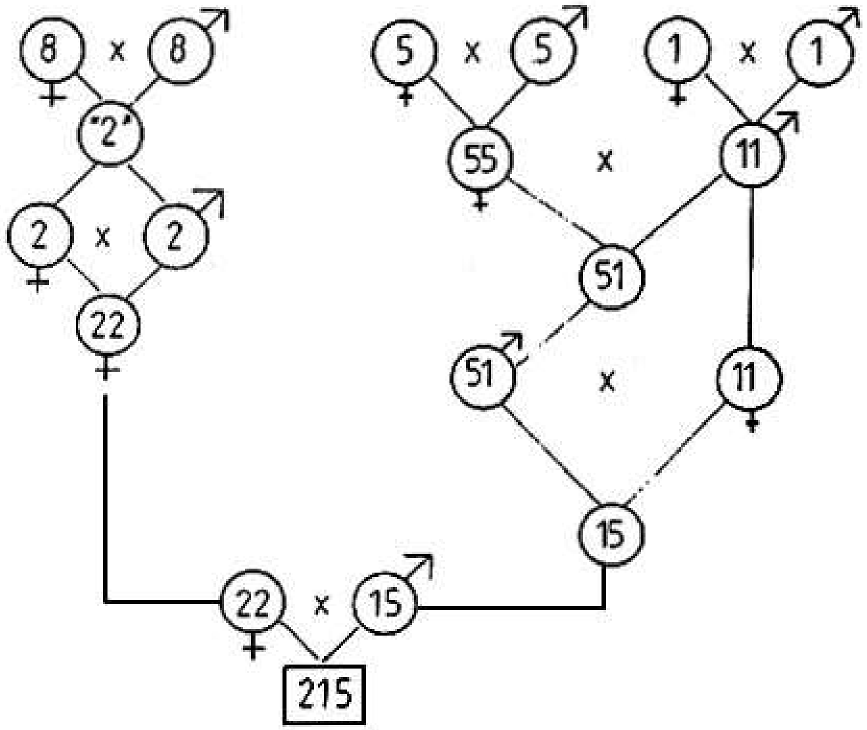


# Hybrid carp lines in hungary1.

**Breeding strategy of the Szarvas 215 hybrid mirror carp .**
**Three breeds were used.**

Anyai vonal

Apai vonal



1967 1. Generáció

I szelekció

1972 2. Generáció

II szelekció

1977

1962 / 1963

1965

hibridizáció

1972

visszakeresztezés

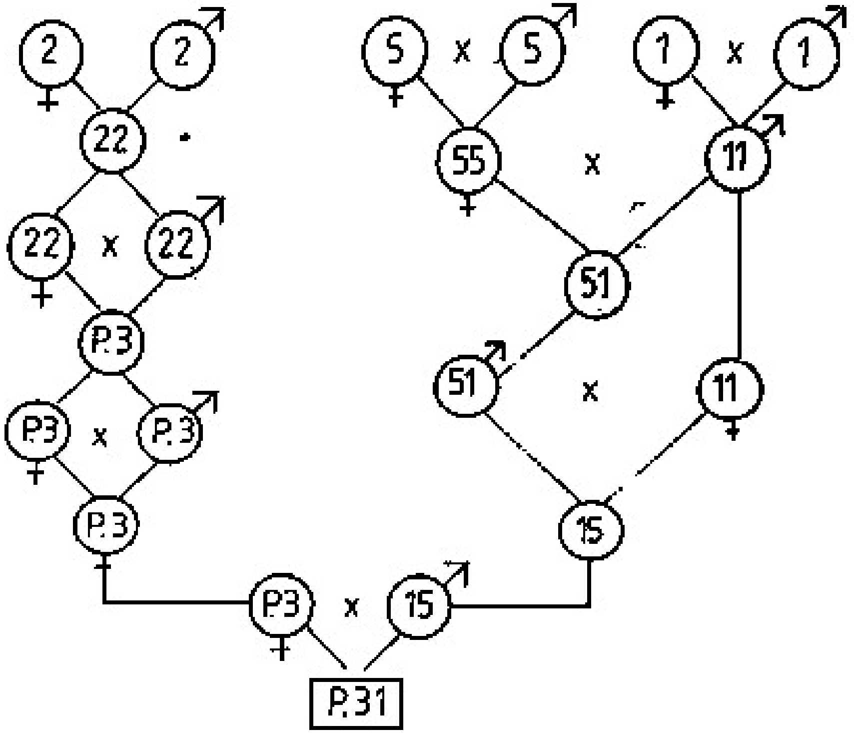
More than three generations and 15 years (generation time ~4 years)

# Hybrid carp lines in hungary2.

**The breeding strategy of the Szarvas P.31 hybrid scalled carp.**
**Three breeds were used.**

Anyai vonal

Apai vonal



1962 1. Generáció

I. szelekció

1967 2. Generáció

II. szelekció

1974 3. Generáció

III. szelekció

1978

1962 / 1963

1965

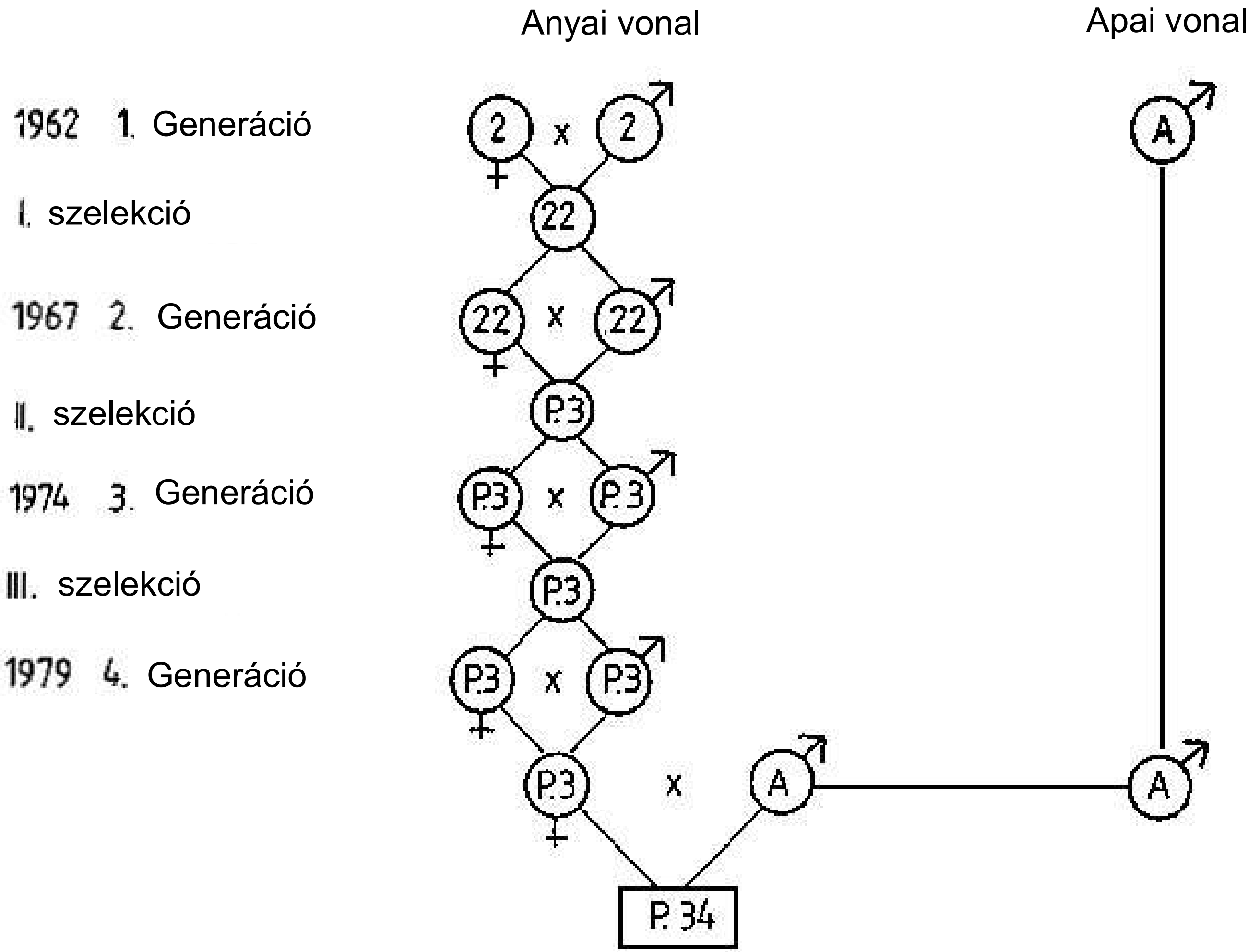
hibridizáció

1972

visszakeresztezés

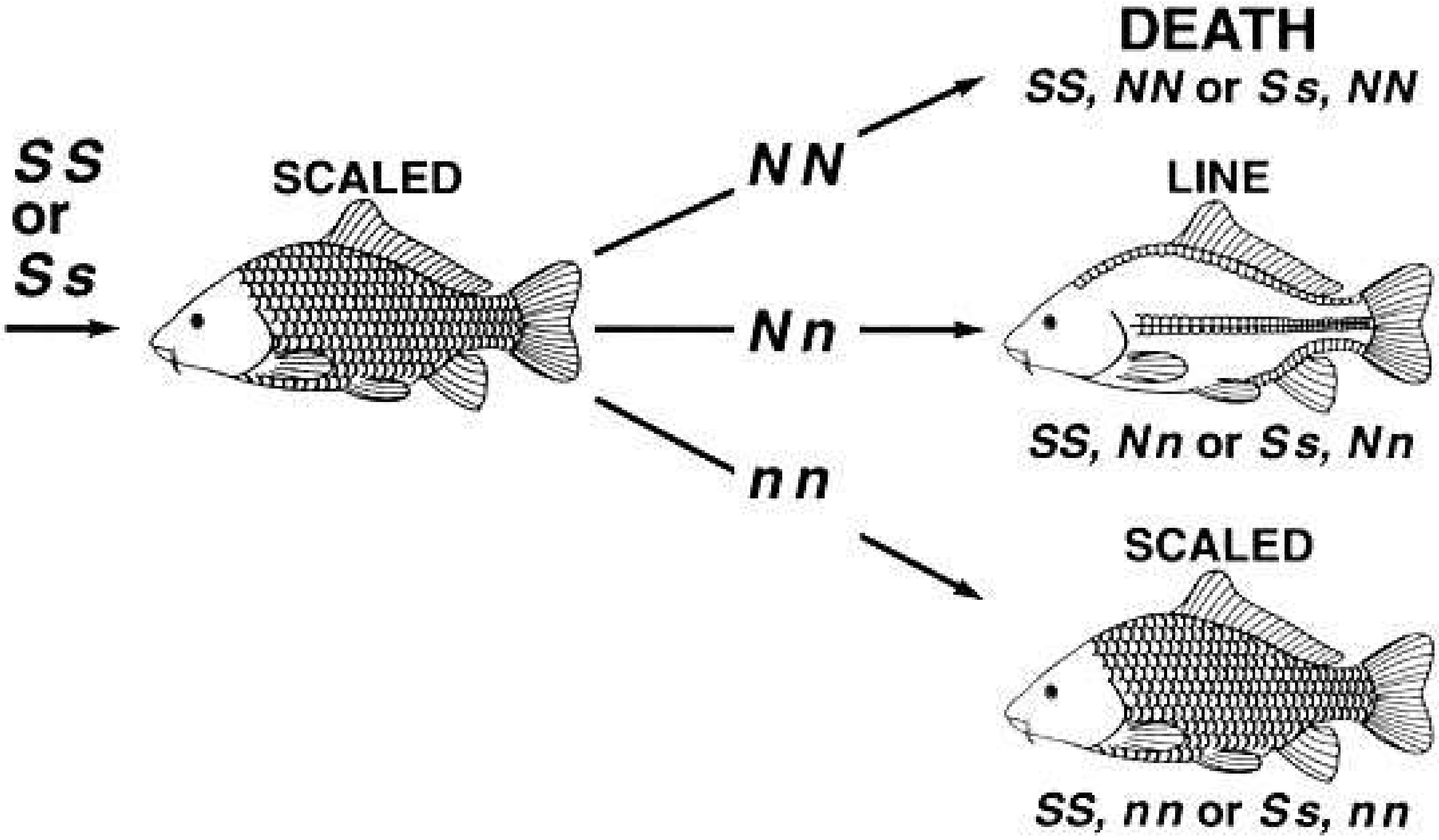
# Hybrid carp lines in hungary3.

**The breeding strategy of the Szarvas P.34 hybrid scalled carp. Cyprinus carpiao carpio subspecies line was selected to cross with Cyprinus carpio haematopterus (Amur) subspecies.**



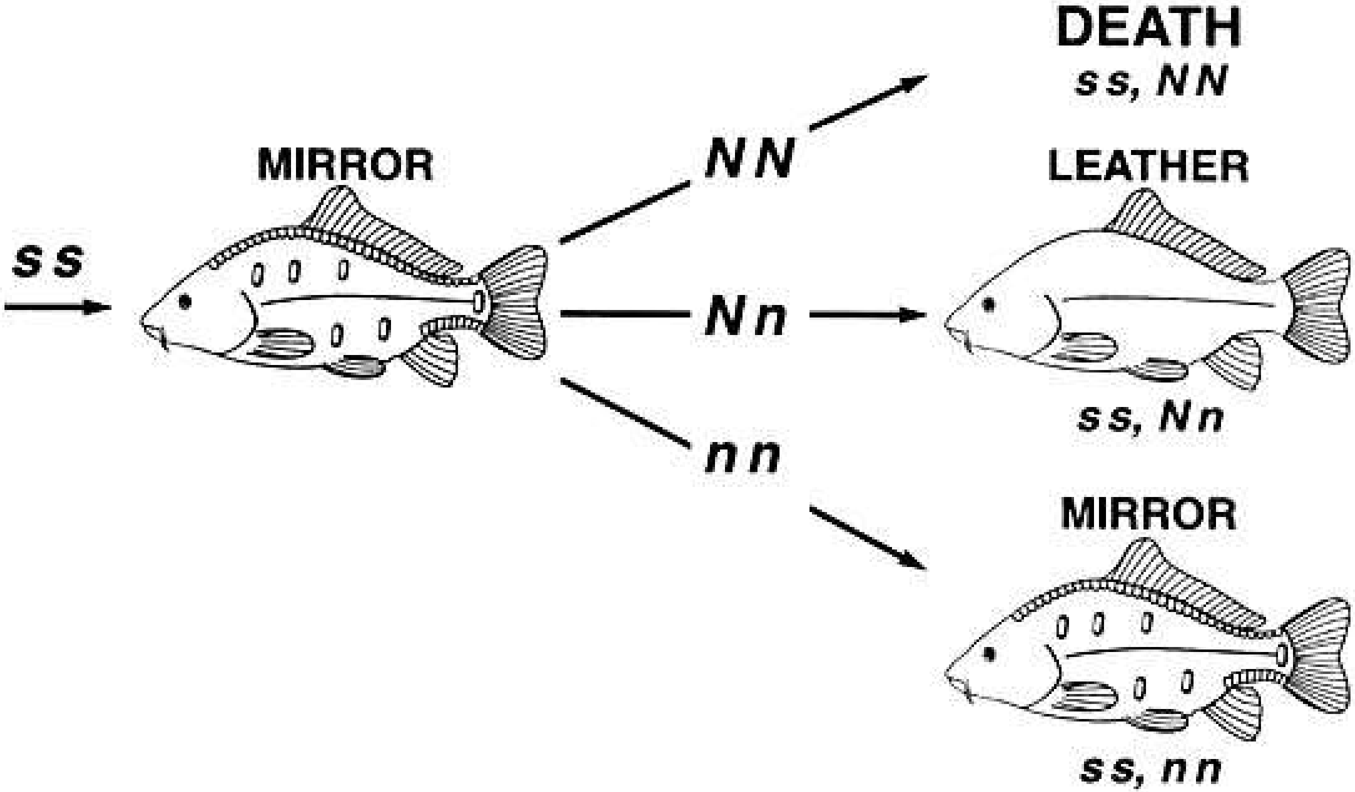
# Cumulateive, Epistaticc, Pleiotropic gene effekt

Scale patter in carp 1



# Cumulateive, Epistaticc, Pleiotropic gene effekt

## Scale patter in carp 2



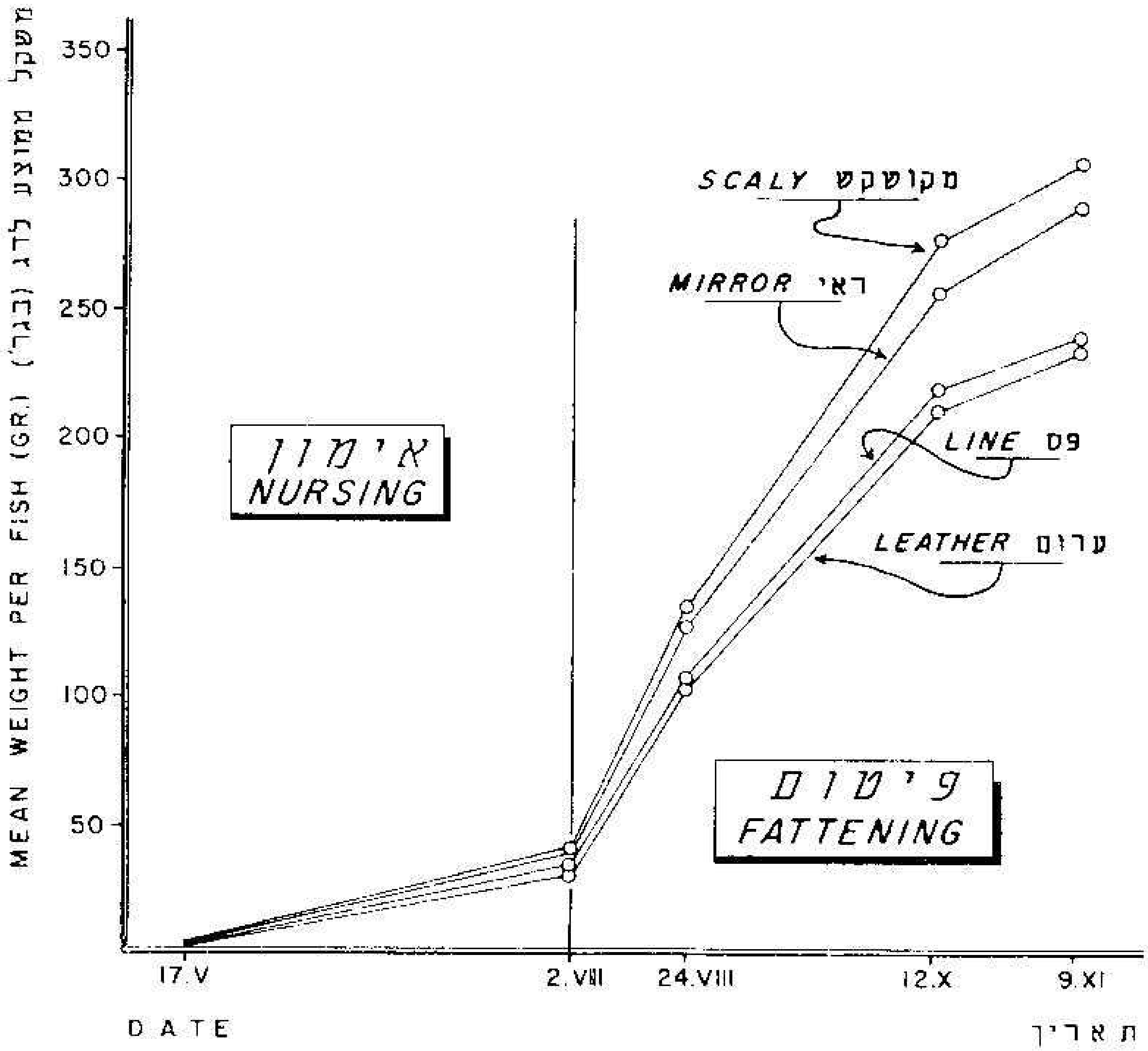


Fig. 3. Growth curves of the four segregated groups of offspring of double heterozygous line carp (Ss Nn) parents.

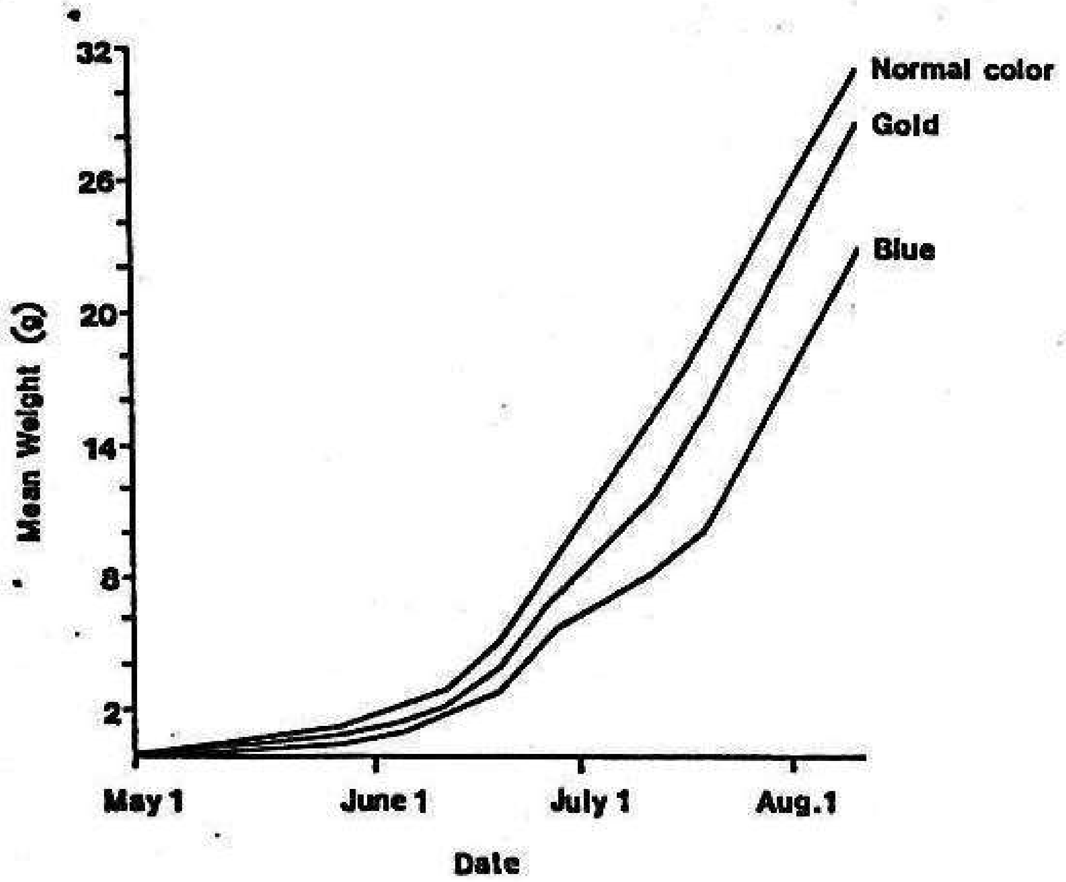


**Figure 3.5** Growth curves of gold, blue, and normally pigmented common carp that were spawned on the same day. The growth curves show the negative pleiotropic effect that the *gg* (gold) and *bb* (blue) genotypes have on growth.
*Source: After Wohlfarth and Moav (1970)*

# Molecular genetics in aquaculture practice

- Markers
- linkage maps
- QTL identification
- Marker assisted selection (MAS)

- FISH barcode – Species identification

- Transgenic fish production

# Genetic markers

**Genetic marker is ………….. that is associated with a genetically determined trait of an organism**

# IMPORTANT TRAITS

Feed conversation
Body weight
Growth rate
Resistance
Cold tolerance
Spawning time
Early surviving rate
Maturity
etc.

Quantitative traits

Determined by several genes

- Different effect levels
- Different type of inheritance

# Genetic markers

Types:

- Phenotypic traits
- Protein polymorphism
- DNS polymorphism

Molecular genetics markers

# Phenotypic markers

Requirements:

- ❖ Unambiguous appearance
- ❖ Not influenced by the environment
- ❖ Determined by a single or a few genes
- ❖ Mendelian inheritance

Problems:

- Only a few phenotypic traits fit these requirements
- More genotypes can produce the same phenotype
- Economical  importance is limited

# Phenotypic markers

Rainbow trout– blue skin color– more varieties

Yamazaki 1974- Japan (cobalt blue)
- Obesity
- liver and kidney degeneration

Kincaid 1975- Great Britain (iridescent metallic blue)
- Increased growth rate

Blanc, 2006- France (cobalt blue)
- 25% production deficit
- Lower survival rate
- Smaller body size

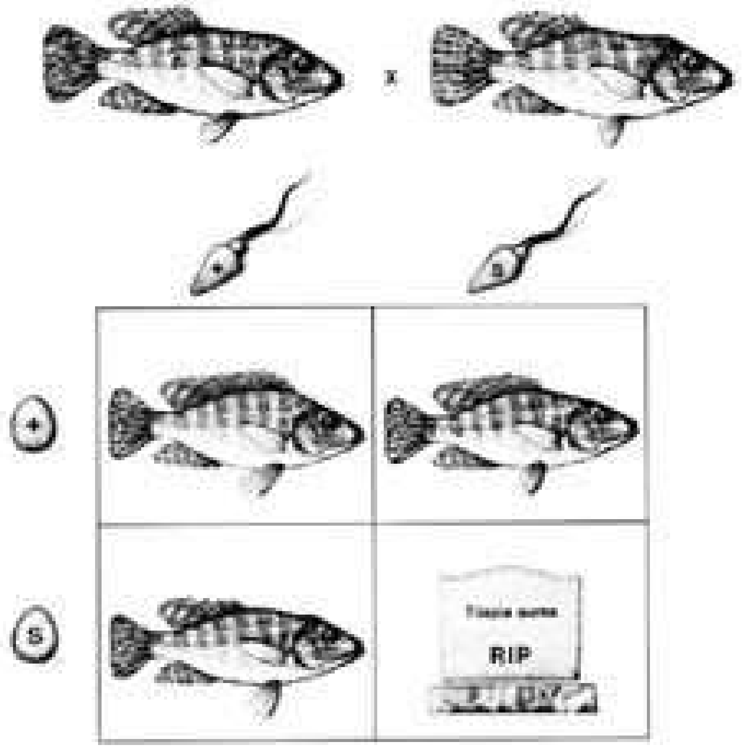
# Phenotypic markers

**_Oreochromis aureus_-Saddleback**

(Tave *et al.* 2006; Auburn University , Israel)

© Noel M. Burkhead

- Dominant phenotype (Homozygous form is lethal)
- Heterozygous form
  - dorsal fin deformation
  - reduced survival rate (3 months 67%)
  - stress sensitivity



# Protein polymorphism

Advantages:

- The developed system can easily be adapted to new species

Disadvantages:

- 50-60 isoenzyme systems are known
- Tissue specificity
- Low number of alleles (2-6)

- Time consuming

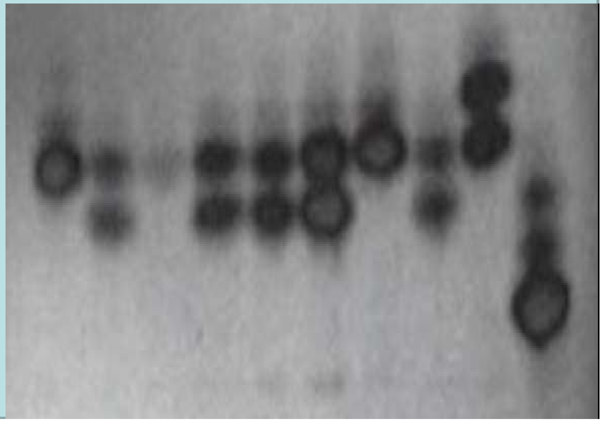
- Difficult to evaluate

Stress sensitivity
(metal, pesticides ,heat ,salt)

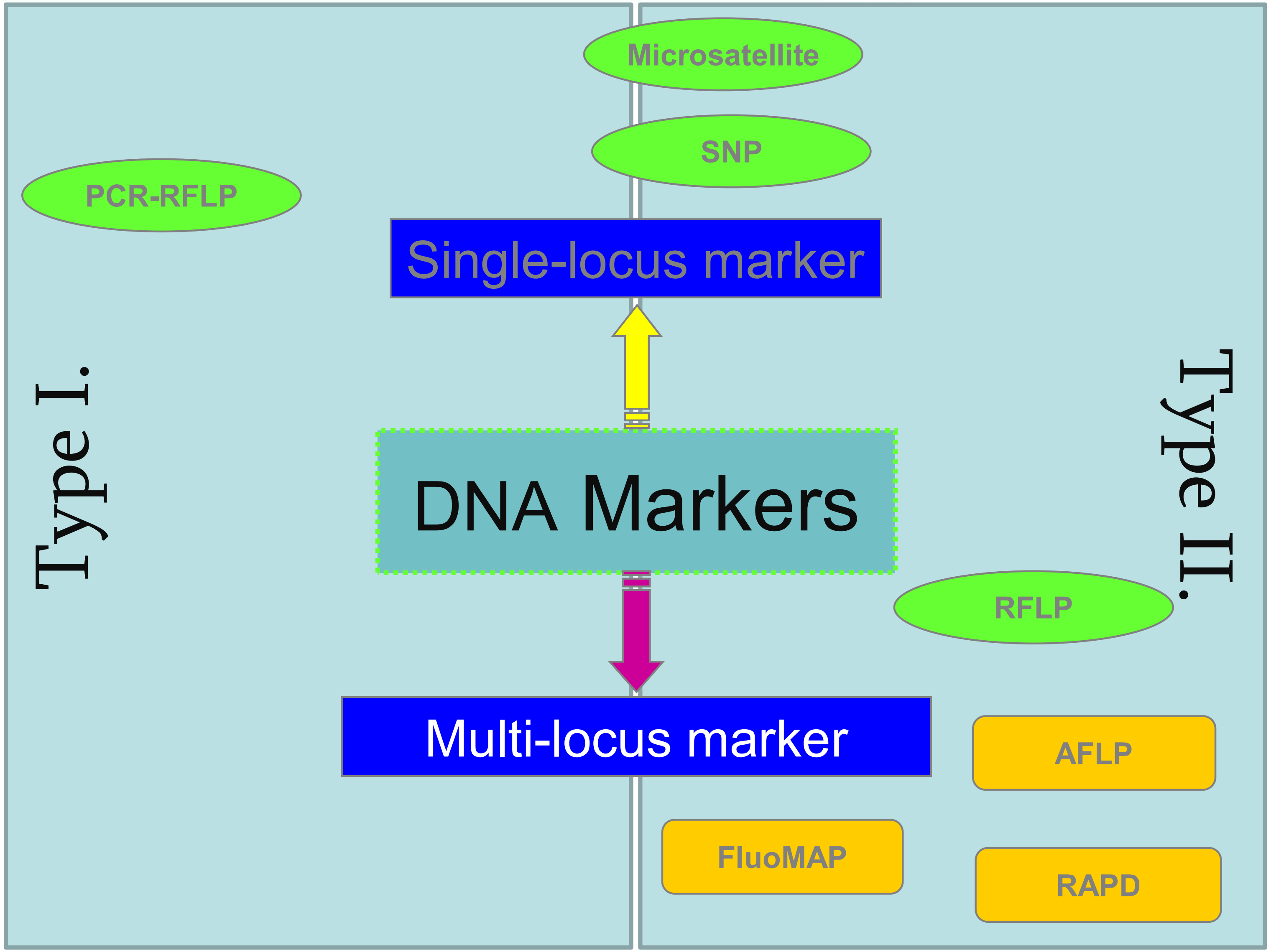
*Gambusia holbrooki*

Glucose-6-phosphate isomerase
Malate dehydrogenase

(Lewis 2001)



# DNA Markers


Microsatellite
SNP
PCR-RFLP
Single-locus marker
Type I.
DNA Markers
Type II.
RFLP
Multi-locus marker
AFLP
FluoMAP
RAPD

# Miniszatellite (DNA fingerprint, RFLP) I.

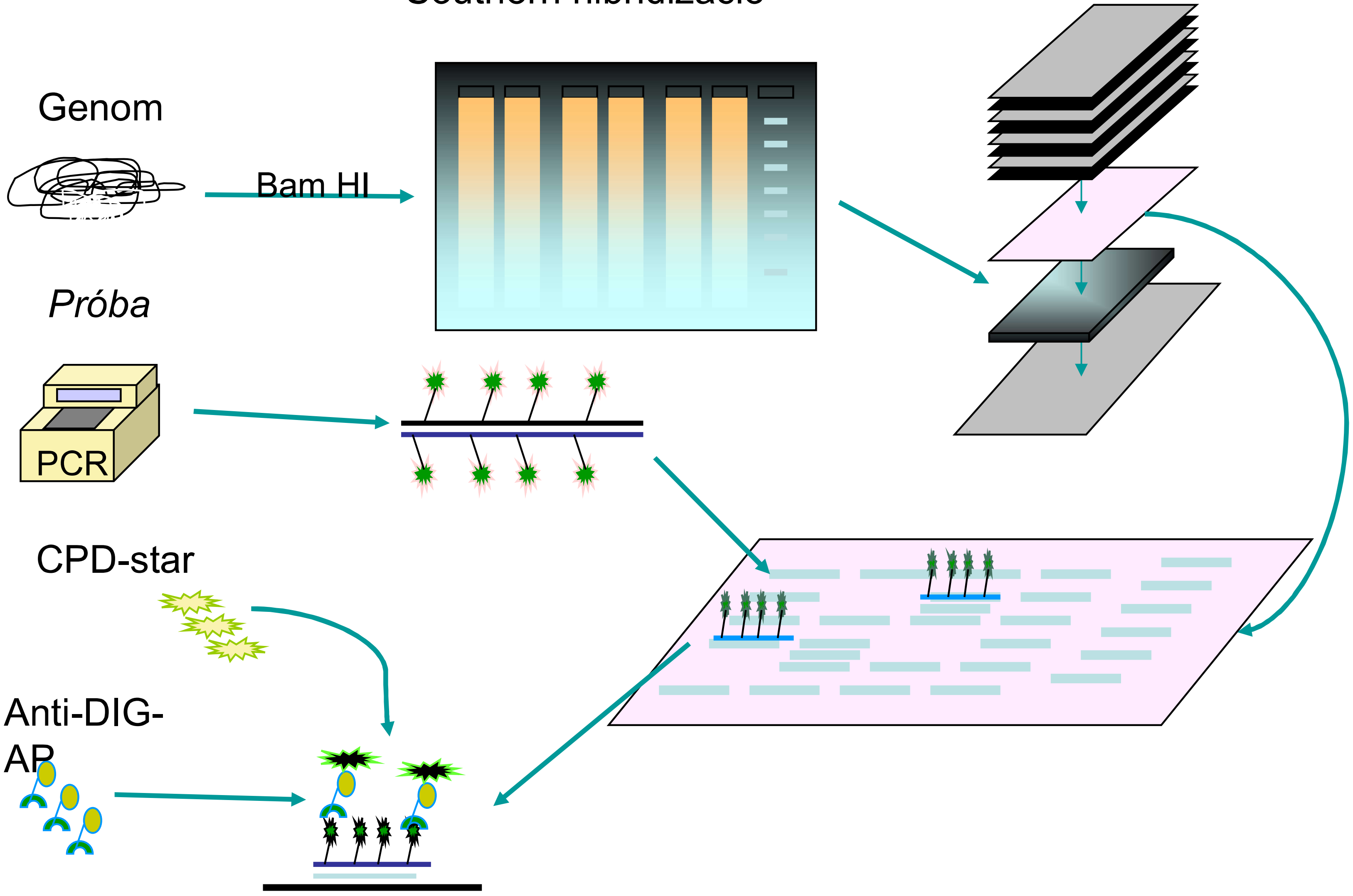
- 16-64bp-os repeats (10-15bp core sequence)
- Recombination hot points

- Detection:
  - Genomic DNA digestion by restriction endonucleases.
  - Agaróz gel electrophoresis (no visible fragments)
  - Transfer and link to nylon membrane
  - Hybridizations with the labelled (radio active or enzymatic) core sequence
  - Evaluation of the fragment patterns

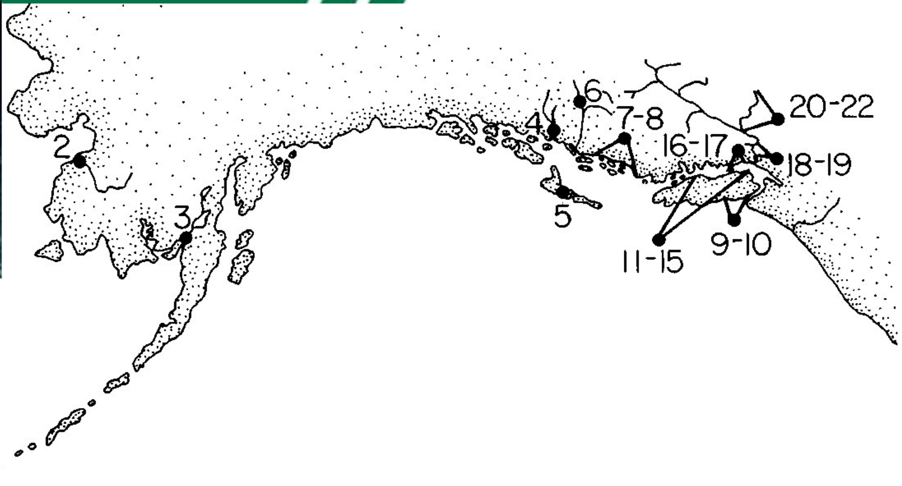
# Miniszatellite (DNA fingerprint, RFLP) II.

- Large amount of tissue / DNA necessary
- Long process
- Difficult fragment patters
- The evaluation contain mistakes
- Repeats are „rear” (1500kb/1 repeat)
- Repeats are present at the ends of the chr.-s.

# Southern hibridizáció



# Miniszatellite- Coho salmon (*Oncorhynchus kisutch*)



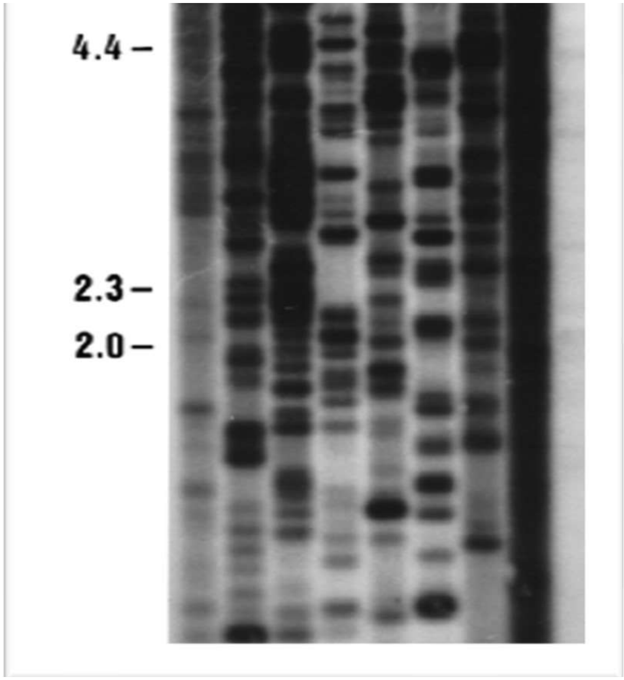
22 population
50 individual/ population

Ssa1 DNS prob

5 restriction enzyme

Only the 45-82% of the individuals were linked to one population.

(Miller 1996 Can. J. Fish. Aquat. Sci. 53: 181–195)



# Mikroszatellite marker

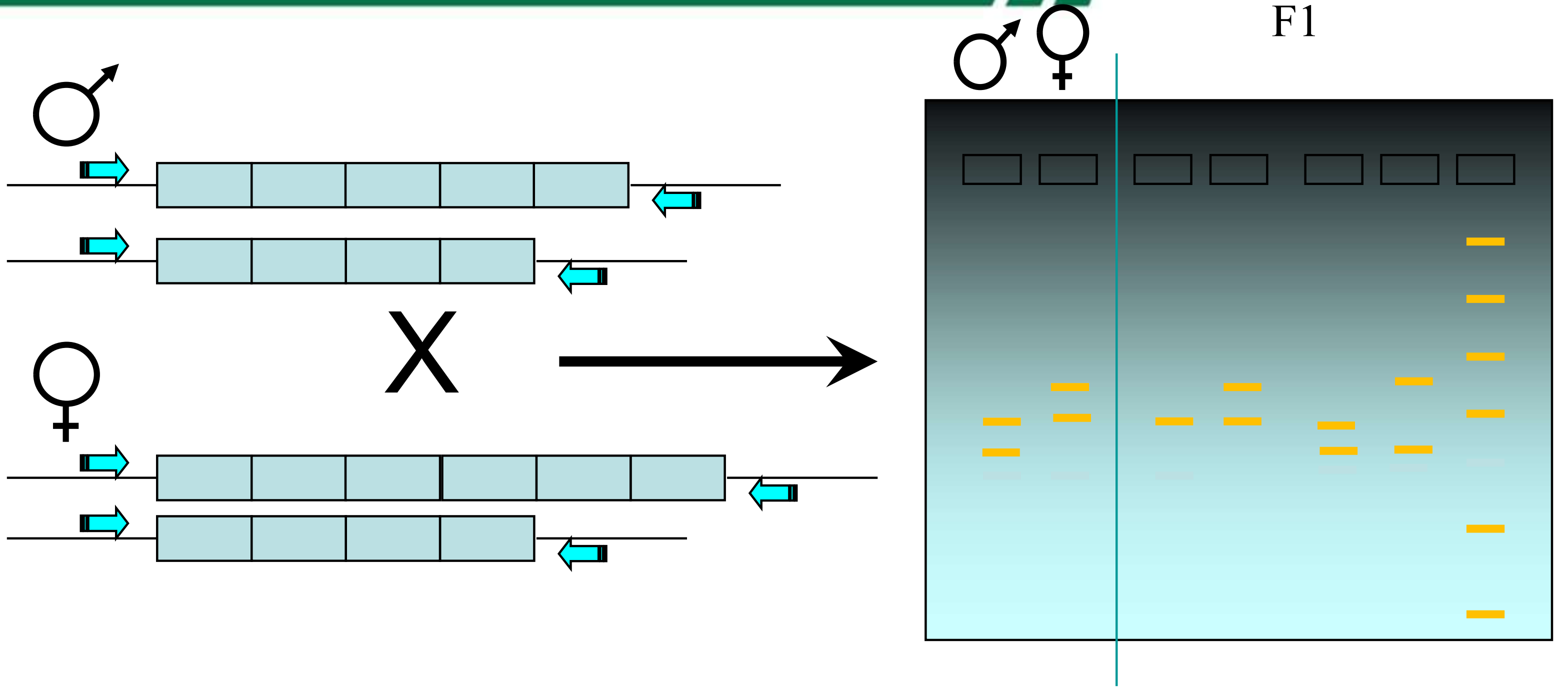
- 1-6 bp repeat sequences
- Common in every genome
- I the fish genomes every 10 Kb DNA contain one microsatellites
- Simple PCR based analyses.

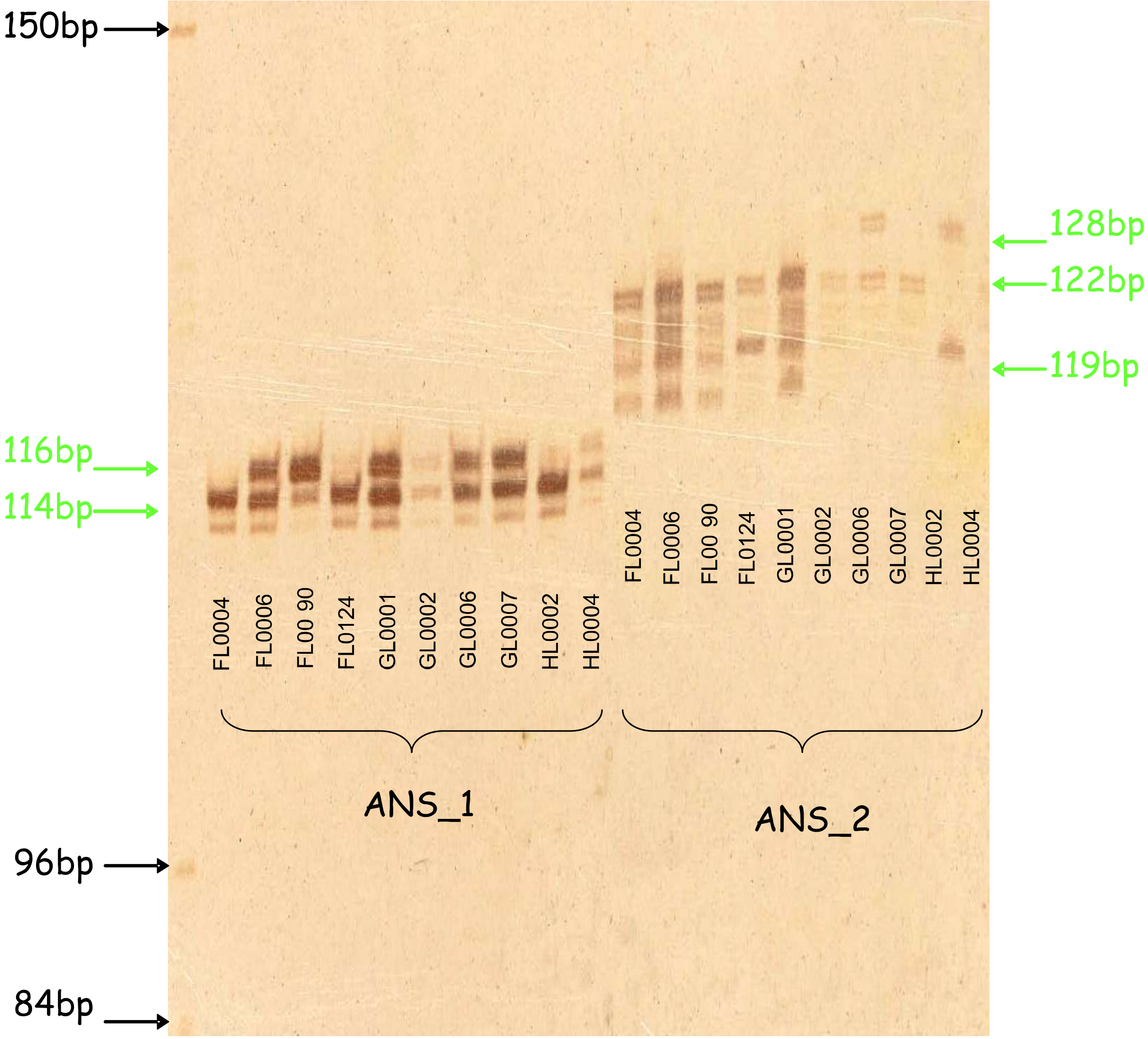
  The primers hybridize to the flanking sequences.

  the fragmet size 50-300bp ( in fish are usually longer)

- Fragment size detection:
  - polyacrylamide gel (radioactive labelling, or silver staining),
  - Special agarose gel (ethidium bromide)
  - Capillary electrophoresis(fluorescens)

# Microsatellite




150bp
128bp
122bp
119bp
116bp
114bp
FL0004
FL0006
FL00 90
FL0124
GL0001
GL0002
GL0006
GL0007
HL0002
HL0004
FL0004
FL0006
FL00 90
FL0124
GL0001
GL0002
GL0006
GL0007
HL0002
HL0004
ANS_1
ANS_2
96bp
84bp

# Mikroszatellit markerek II.

- Advantages:
  - Small amount of DNA necessary
  - Fast
  - Codominant (homo- and heterozygot genotypes)
  - High nuber of alleles (25-30 allel/population/microsatellite)
  - The marker can be adapt to close relarive species

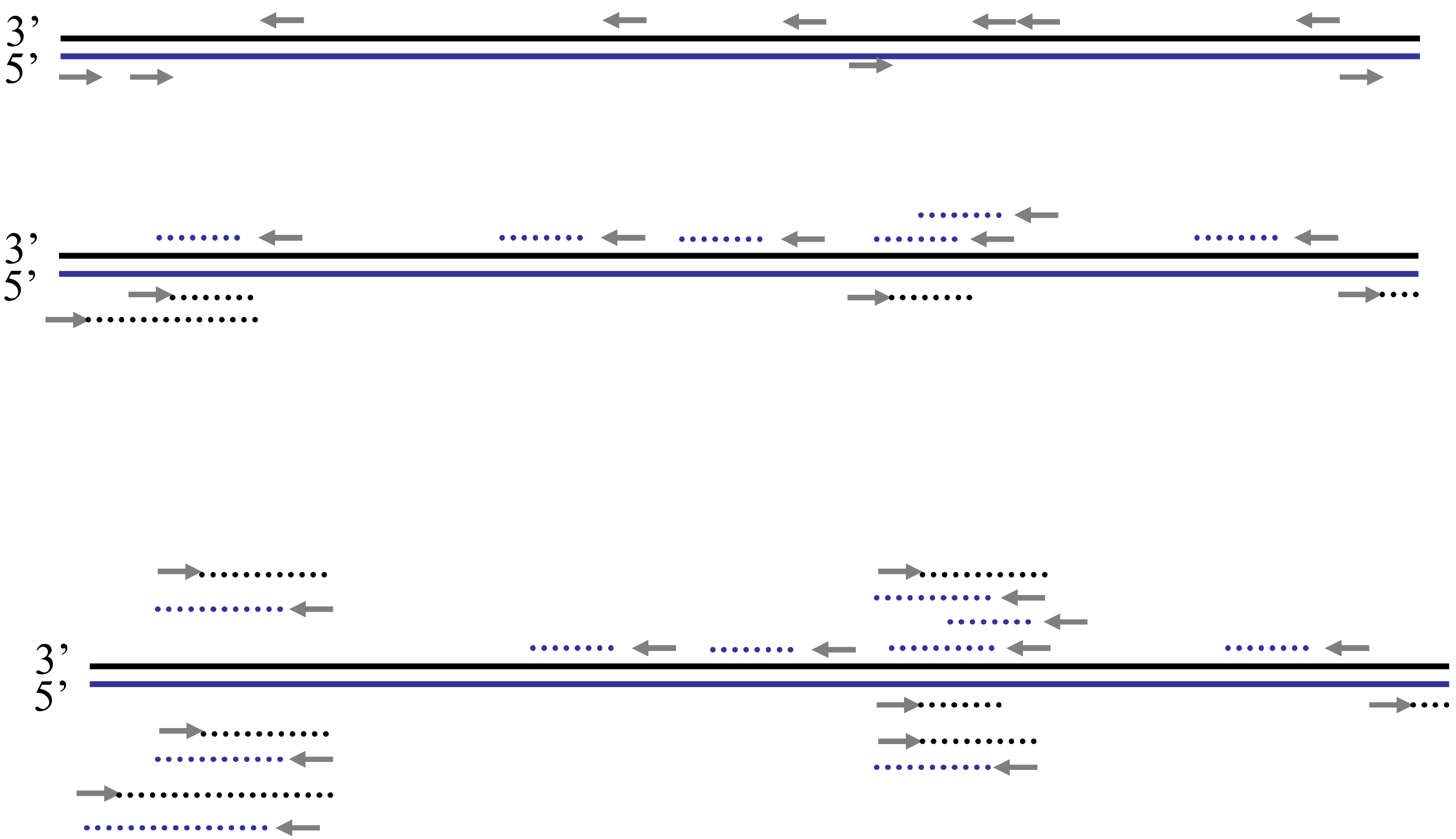
- Disadvantages:
  - Sequence information necessary
  - High price of the marker development
  - analyze only one point of the genome (multiplexing possible 2-3 marker and four different fluorescents dye)

# Random Amplified Polymorphic DNA (RAPD)

- Williams et al., 1990; Welsh and McClelland, 1990;

- Short 6-12bp long primers
- Amplify more fragments (3-30) in one reaction.
- 100bp-3500bp fragment range
- Most fragments are monomorphic (present in all individuals), others are polymorph ( not present in all individuals - These are informative)

# RAPD



# RAPD I.

Advantages:

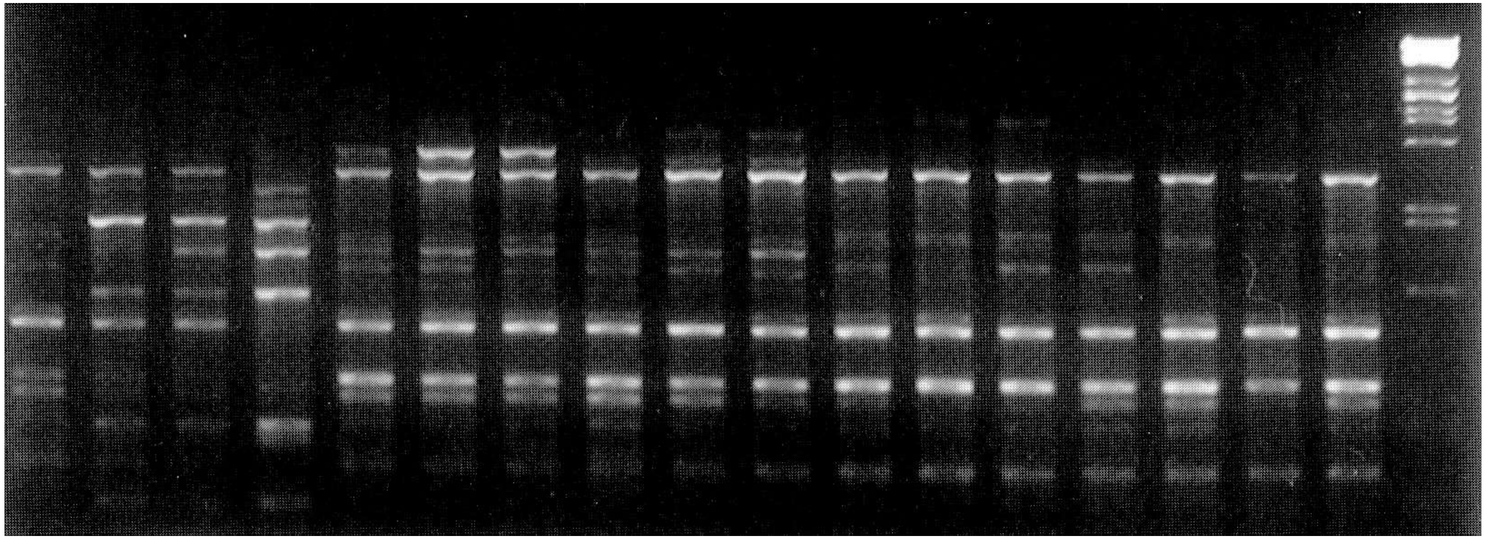
- Can be used for analyses of unknown genomes
- It is working on any species
- Very cheap
- Primer sets can be by from suppliers
- Fast and simple
- Different fragment patterns can be generated with combination of two primers or restriction enzymes

# RAPD II.

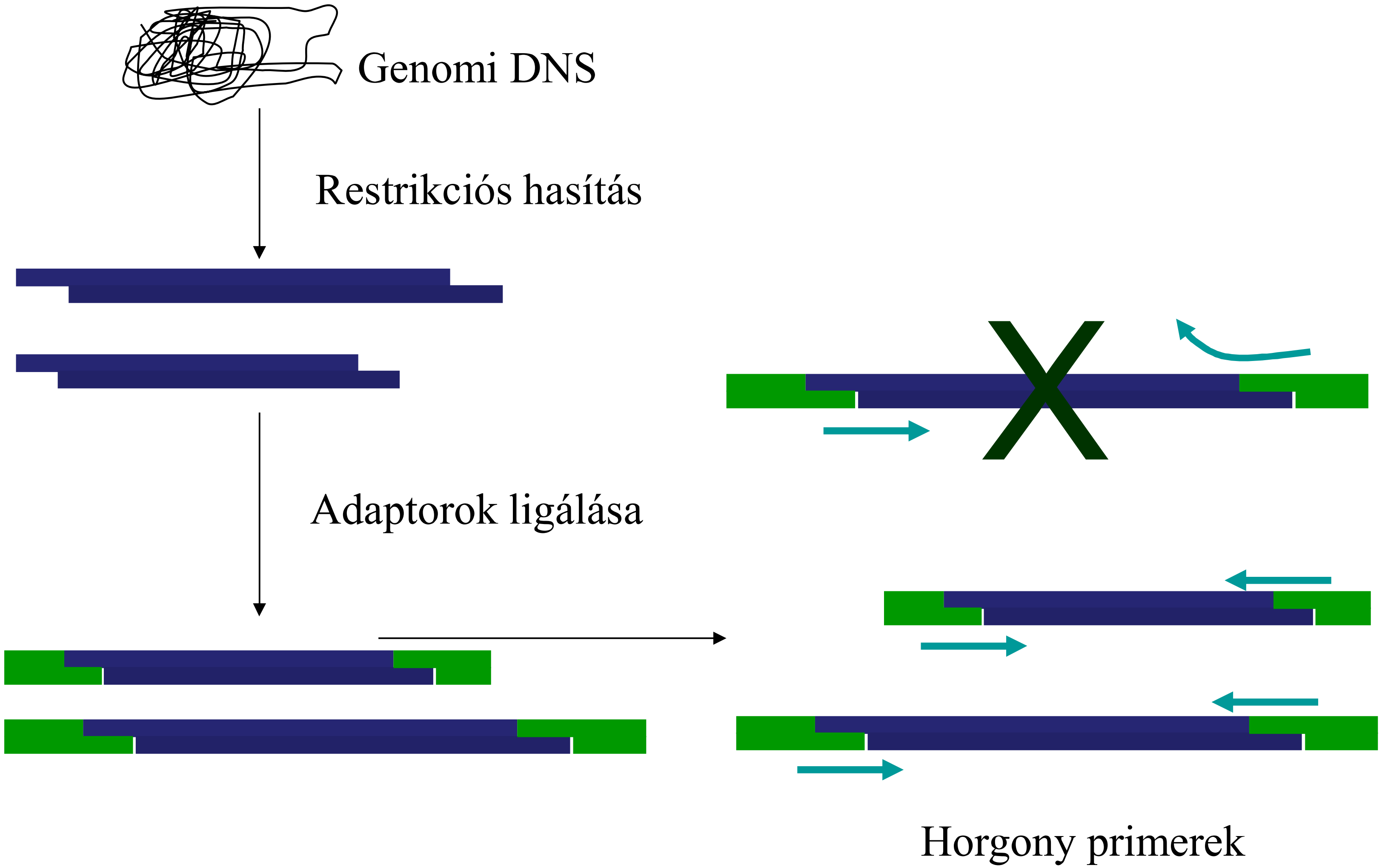
Disadvantages:

- Very sensitive for reaction conditions
- Dominant- recessive inheritance of the fragments
- The origin and the relationship of the fragments are not known

# RAPD analízis



# AFLP



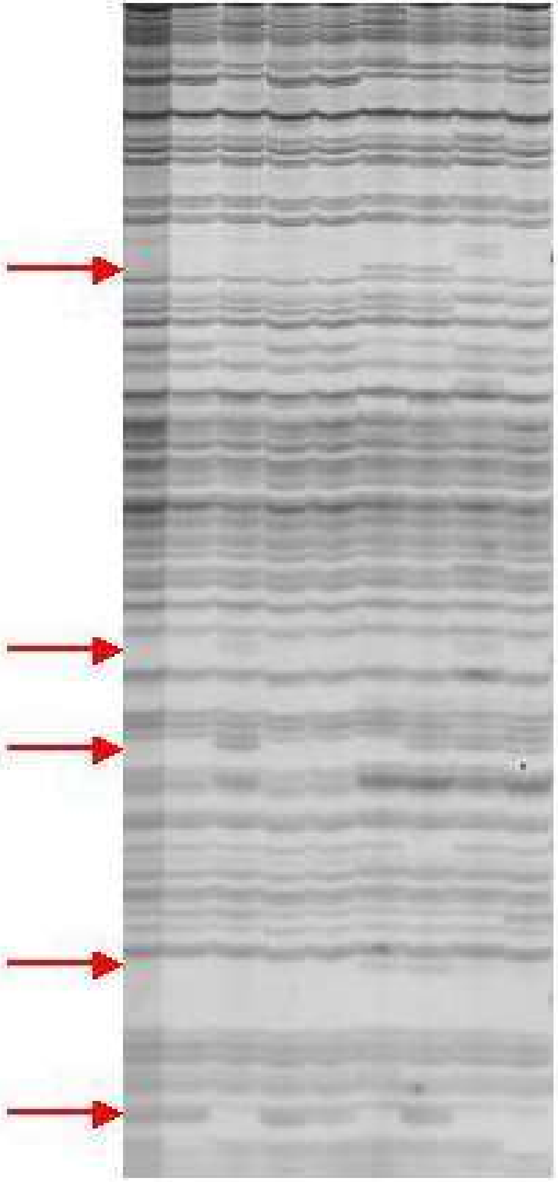
Genomi DNS

Restrikciós hasítás

Adaptorok ligálása

Horgony primerek

# AFLP



# AFLP I.

Advantages:

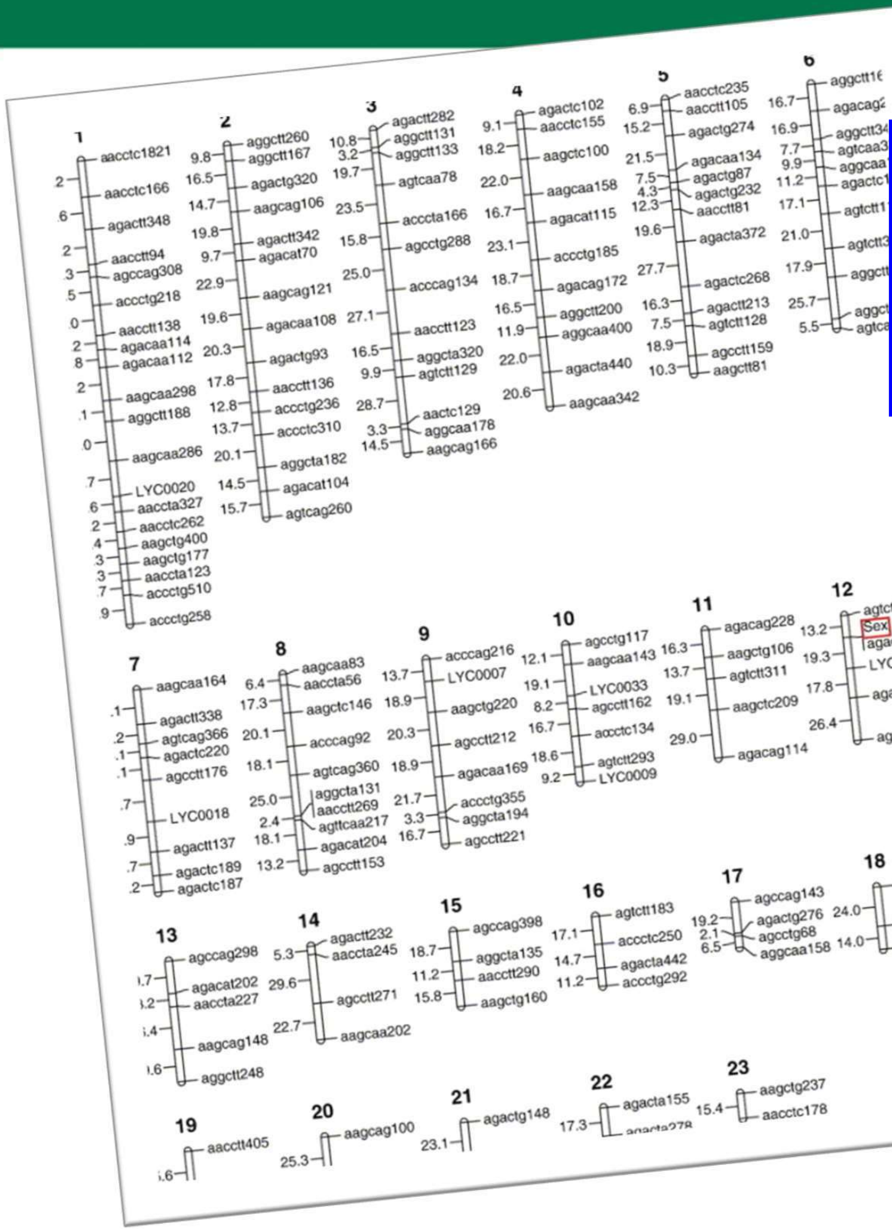
- Can be used for analyses of unknown genomes
- It is working on any species
- Primer sets can be by from suppliers
- Not sensitive for reaction conditions
- The evaluation can be automatized

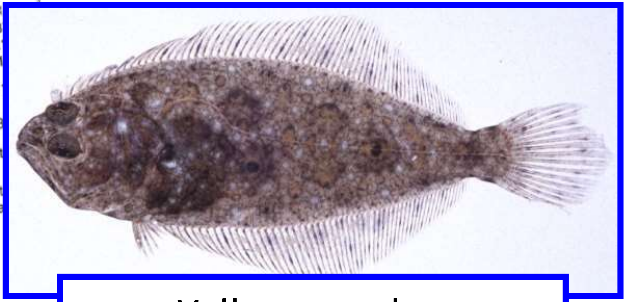
# AFLP II.

Disadvantages:

- Dominant- recessive inheritance of the fragments
- The origin and the relationship of the fragments are not known
- Time consuming
- More expensive

# AFLP





Yellow croaker
( *Pseudosciaena crocea)*

- 153 db AFLP marker
- 94 individuals

Aim:

- Economically important traits (QTLs) can be mapped
- Can be used fore breeding

Yue Ning 2007Aquaculture 264, 1-4, 16-26

# SNP- Single Nucleotide Polymorphism

- Single nucleotide difference

  Types:
  - Non coding
  - Coding
    - Synonymous
    - Non -synonymous

- The all genome
- Sequence knowledge required
- Codominant inheritance

- More than two alleles are rear
- Maximum four alleles

Small information content – 2-3x more SNP marker necessary for analyses than microsatellites

- Time consuming
- Expensive

# SNP- Single Nucleotide Polymorphism

Detection methodes:

PCR based:

One step – High resolution melt analyses

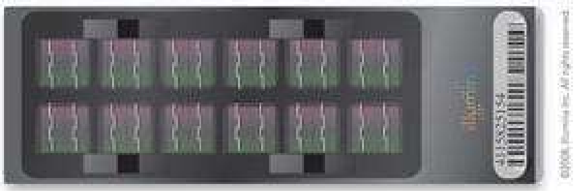
Two step – (first step fragment amplification, second step SNP allele detection)

- Capillary sequencing
- Taqman probe, Flap endonuclease
- single nucleotide extension assay (SNUPE, iPLEX)
- PCR-RFLP
- Single strand conformation polymorphism
- etc.

- slow
- time consuming
- expensive

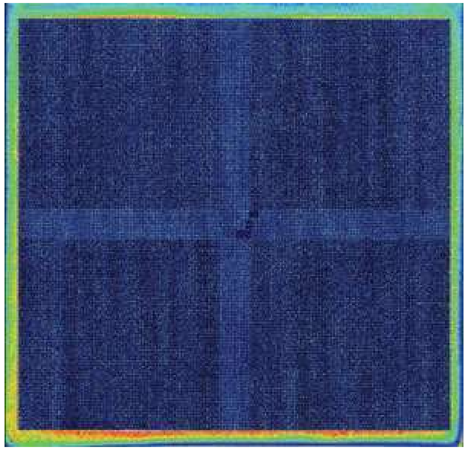
# SNP- Single Nucleotide Polymorphism

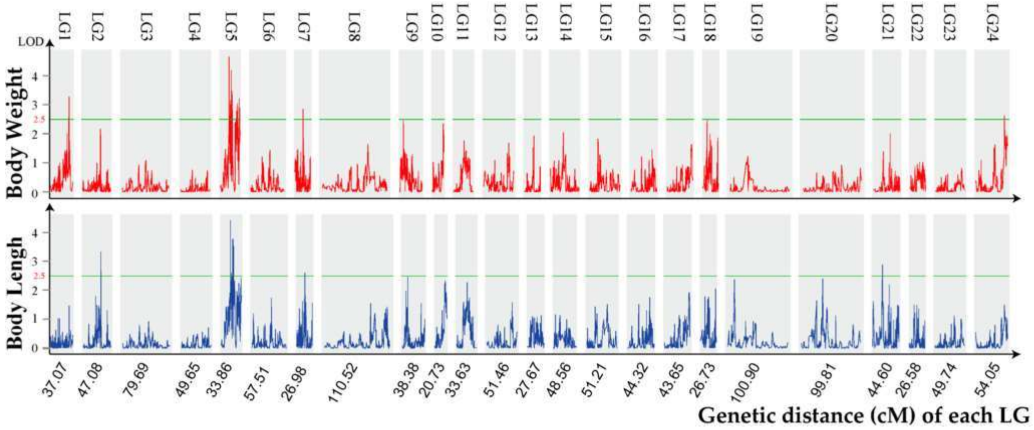
MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM



Detection methods:

- Hybridization based ( Chip)
  - Genome wide analyses
  - Expensive
- NGS based ( RAD, ddRAD sequencing)



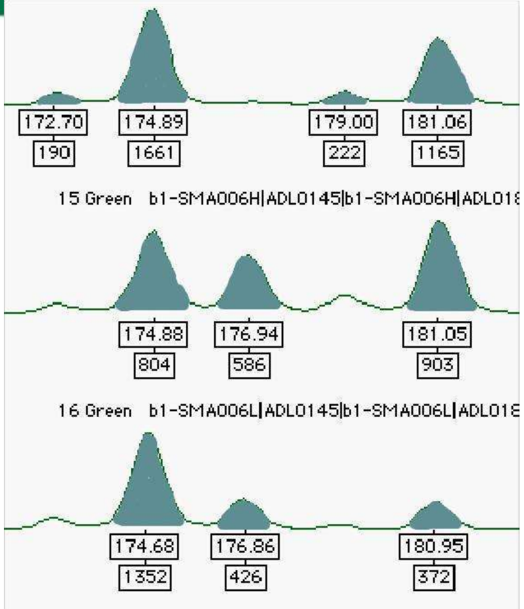


# Properties of Different MM

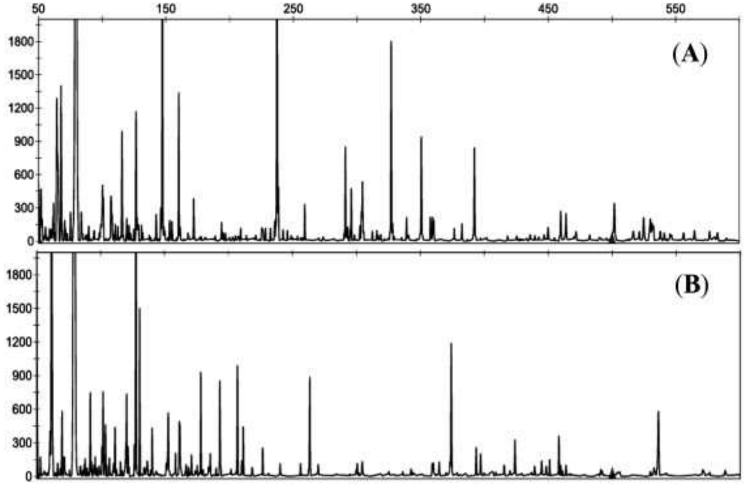
| Features | RFLP | PCR-RFLP | RAPD | FluoMAP | AFLP | Micro-satellite | SNP |
|---|---|---|---|---|---|---|---|
| Preliminary sequence inf. | Yes | Yes | No | No | No | Yes | Yes |
| Detection method | Hybridization | PCR | PCR | PCR | PCR | PCR | PCR |
| Reproducibility | High | High | low | Medium | Medium | High | High |
| Frequency in genomes | Limited | Limited | High | High | High | High | Very high |
| Degree of polymorphisms | Low | Low | Medium | High | Medium to High | High | Low |
| Phenotype expression | Codominant | Co-dominant | Dominant-recessive | Dominant-recessive | Dominant-recessive | Co-dominant | Co-dominant |
| Possibility of automation | No | No/Yes | No | Yes | Yes | Yes | Yes |
| Cost | High | High | Low | Low | Medium | High | High |

# Short Range Applications

- **Parentage determination**
- **Sexing of embryos**
- **Detection of inbreeding , diversities and genetic distance**
- **Confirmation of androgenesis and gynogenesis**
- **Specific marker identification**
  - species
  - subspecies
  - varieties
  - population
  - hybrids



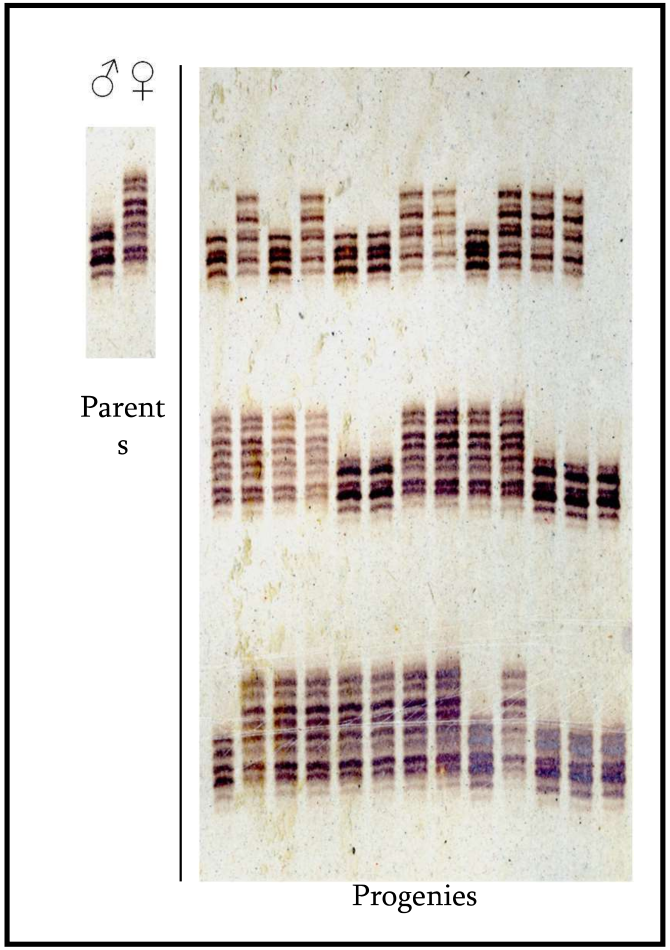
Microsatellite analysis



FluoMAP analysis

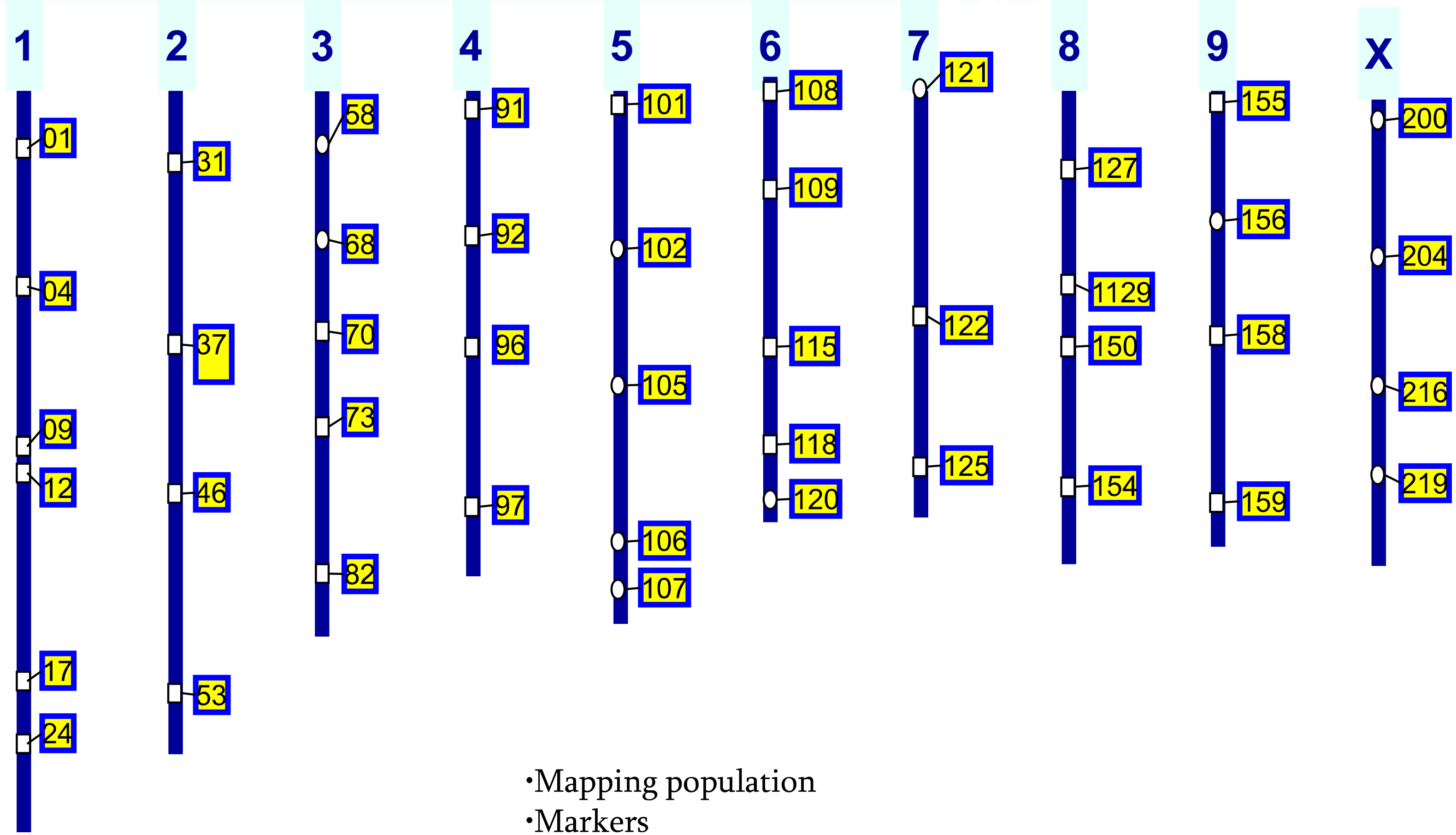
# Long Range Applications

- **Genetic mapping**
- **QTL identification**
- **Marker assisted selection (MAS)**



Microsatellite analysis with PAGE and silver staining

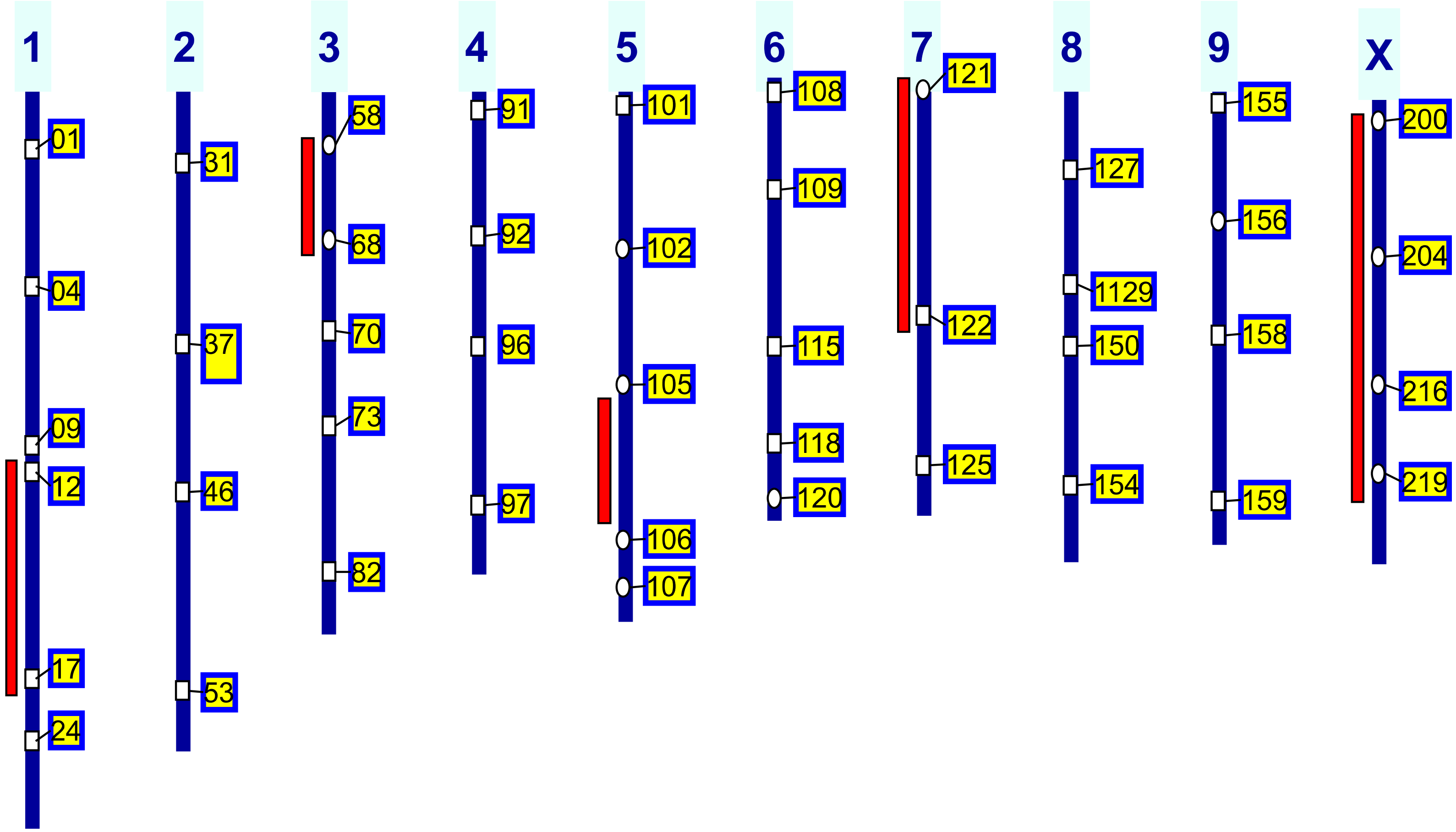
# Linkage maps



- Mapping population
- Markers
- Detection system

# QTL mapsC

MAGYAR AGRÁR- ÉS
ÉLETTUDOMÁNYI EGYETEM

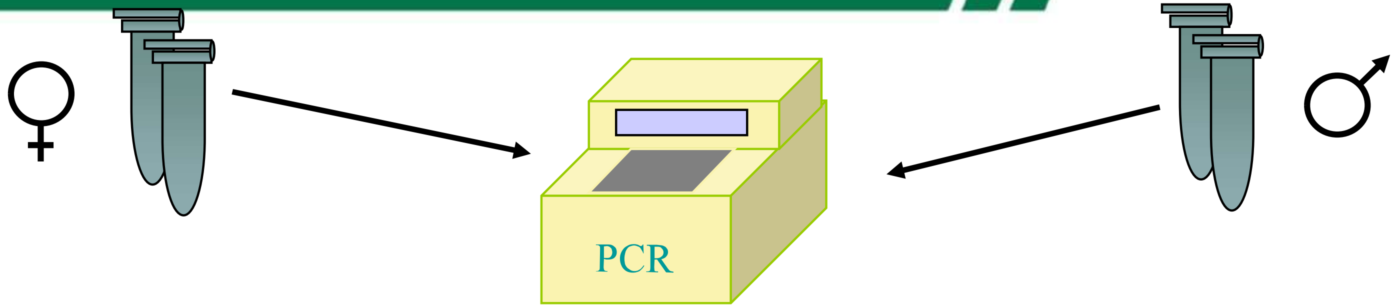


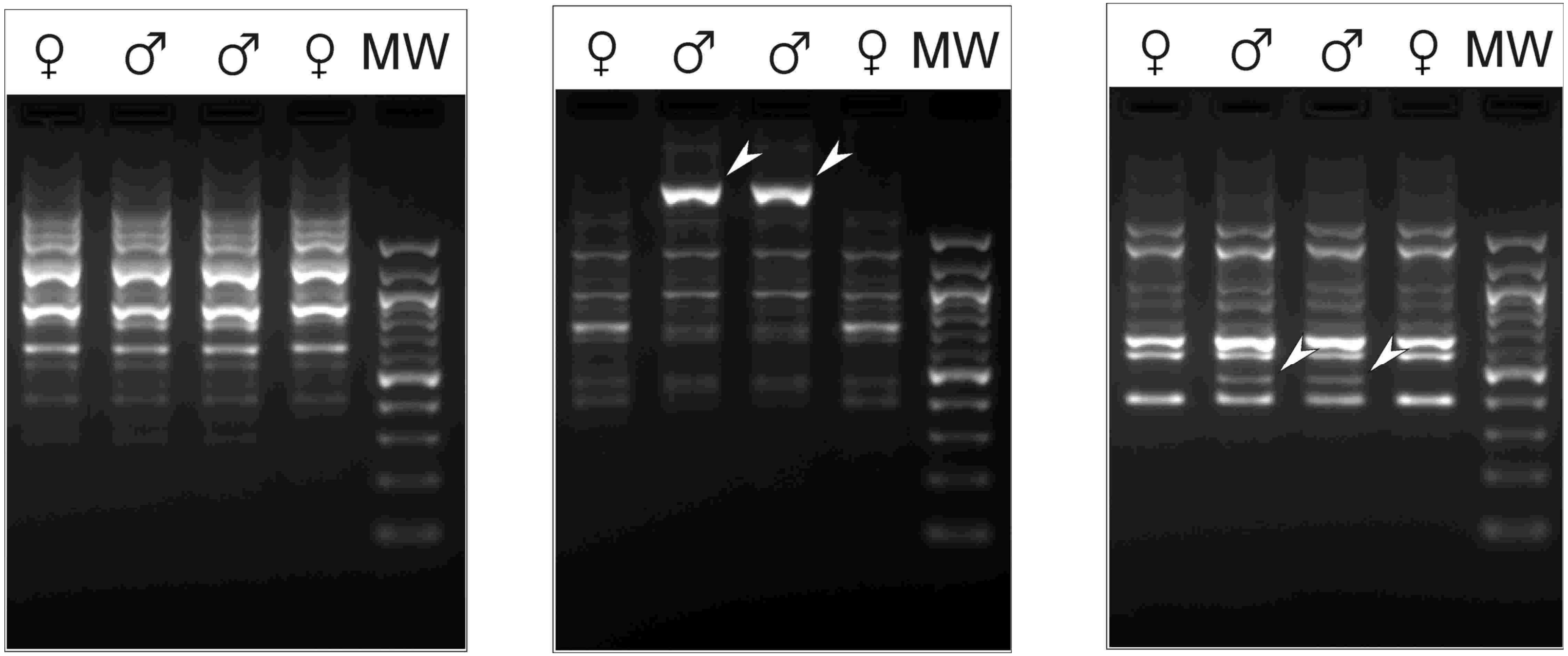
# Applications of DNA markers in aquaculture genetics Tasks, recommended markers

| Tasks | Recommended marker system | Other useful marker types |
|---|---|---|
| **Species identification** | RAPD | AFLP, microsatellites, isozymes |
| **Strain identification** | AFLP, microsatellites | RAPD |
| **Hybrid identification** | RAPD | AFLP, microsatellites, mitochondriaa |
| **Paternity determination** | Microsatellites | |
| **Genetic resource/diversity analysis** | AFLP, microsatellites | RAPD |
| **Genetic mapping** | Type I markers, Microsatellites SNP | AFLP, RFLP |
| **Comparative mapping** | Type I markers | ESTs, conserved microsatellites |

Liu and Cordes (2004) Aquaculture; 238 1 –37

# Sex specific RAPD

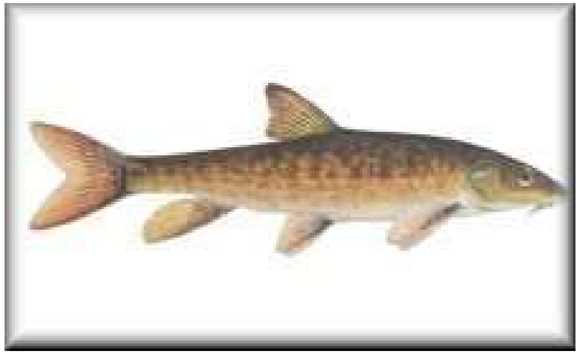


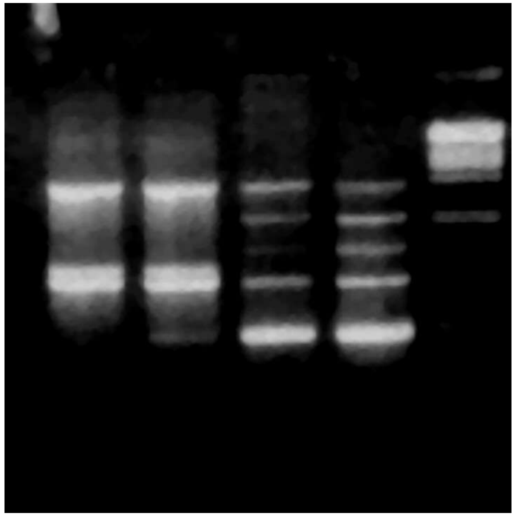


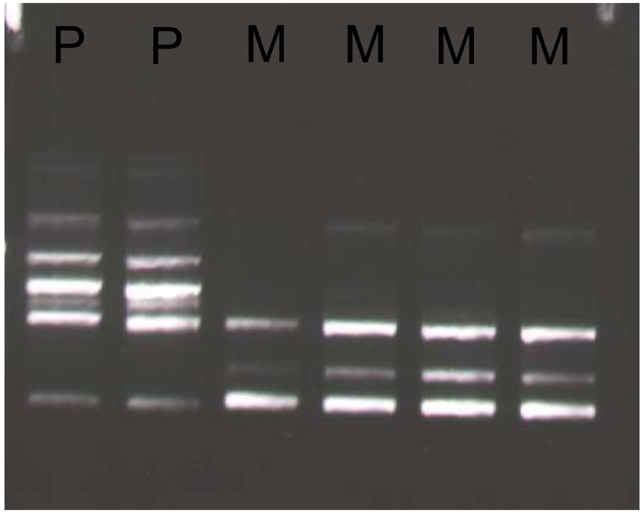
# Species identification

Barbel (*Barbus barbus*)

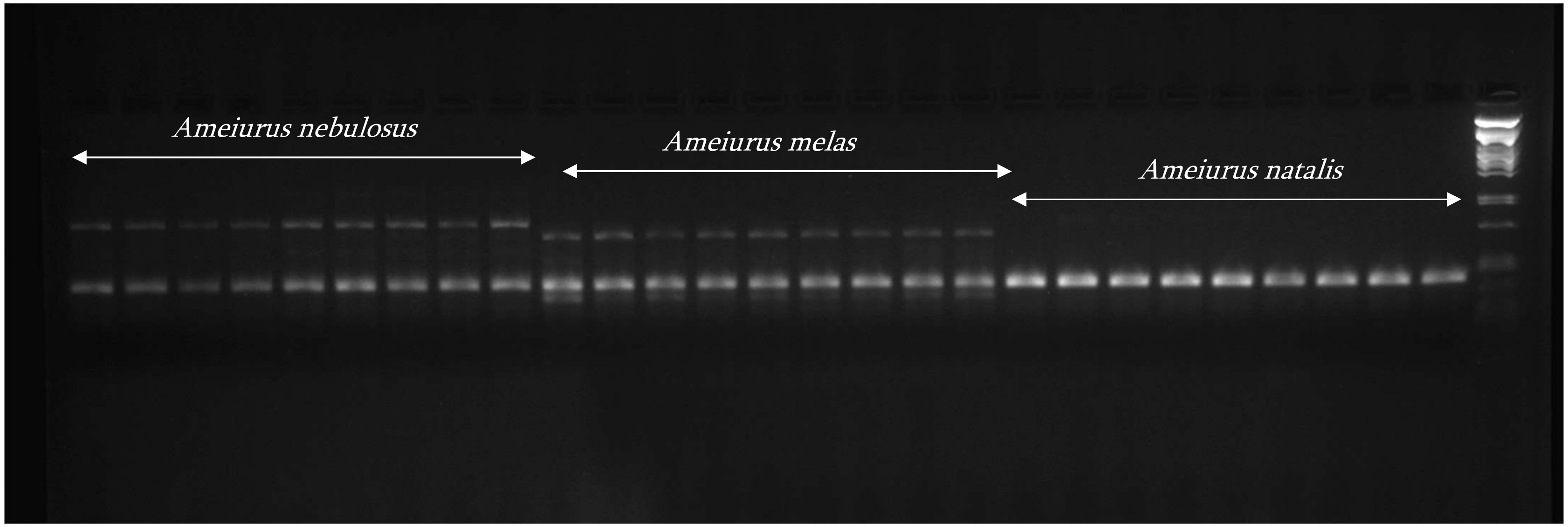
Petényi (Mediterranean) Barbel
(*Barbus meridionalis Petenyii*)



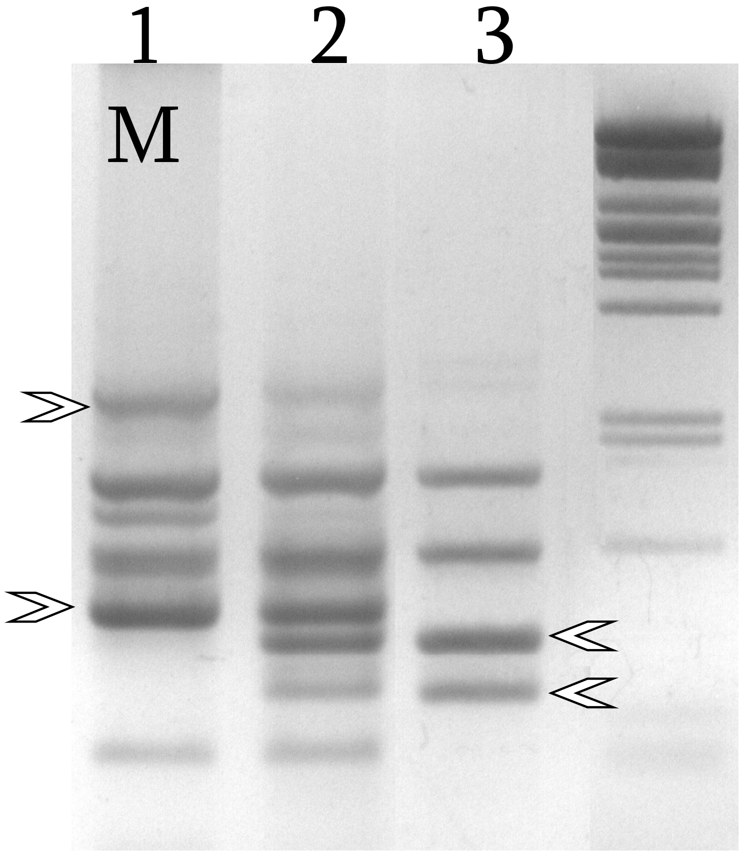


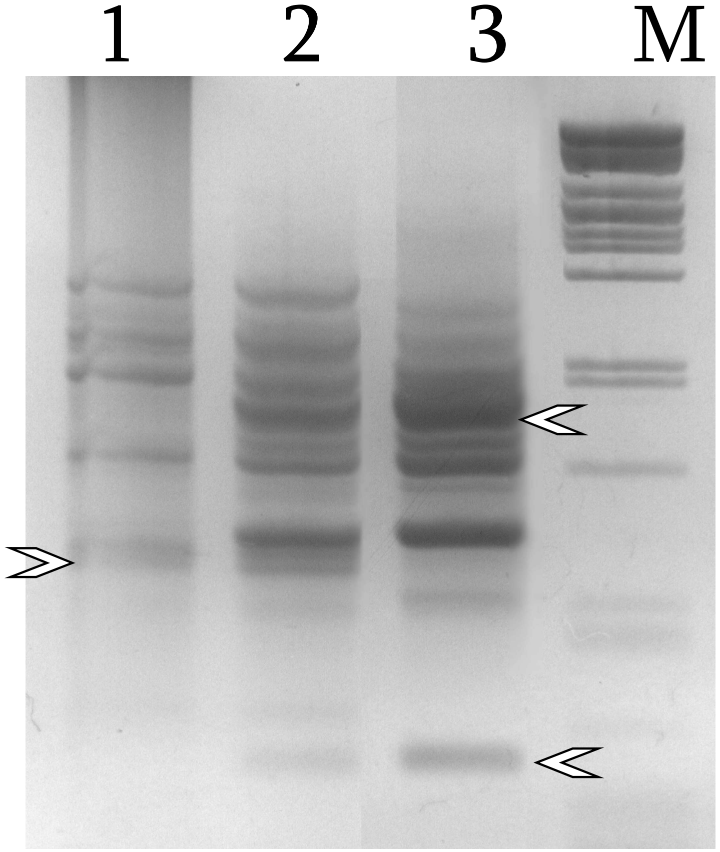


# *Ictalurus spp*. identification

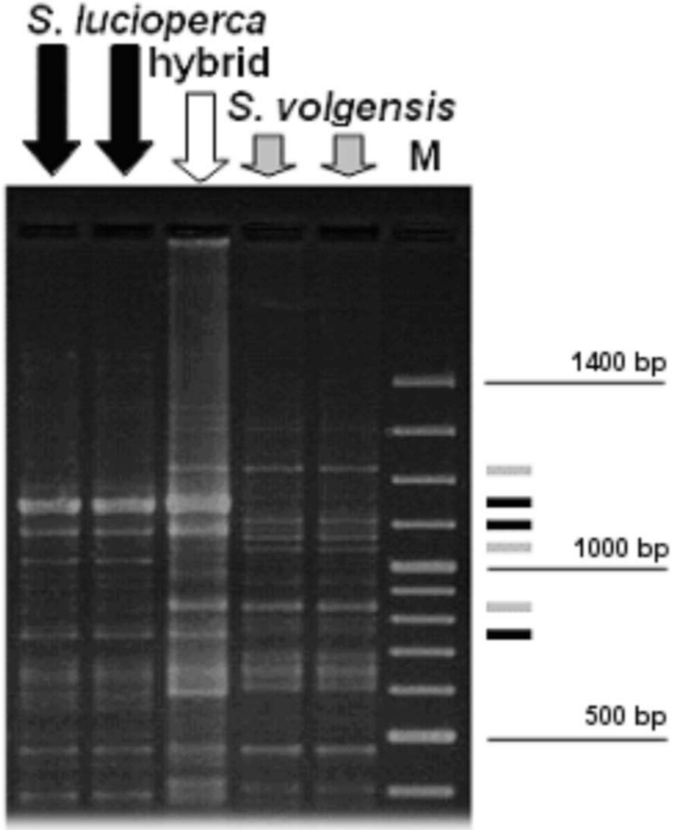


# Sterlet X Atlantic sturgeon hybrid

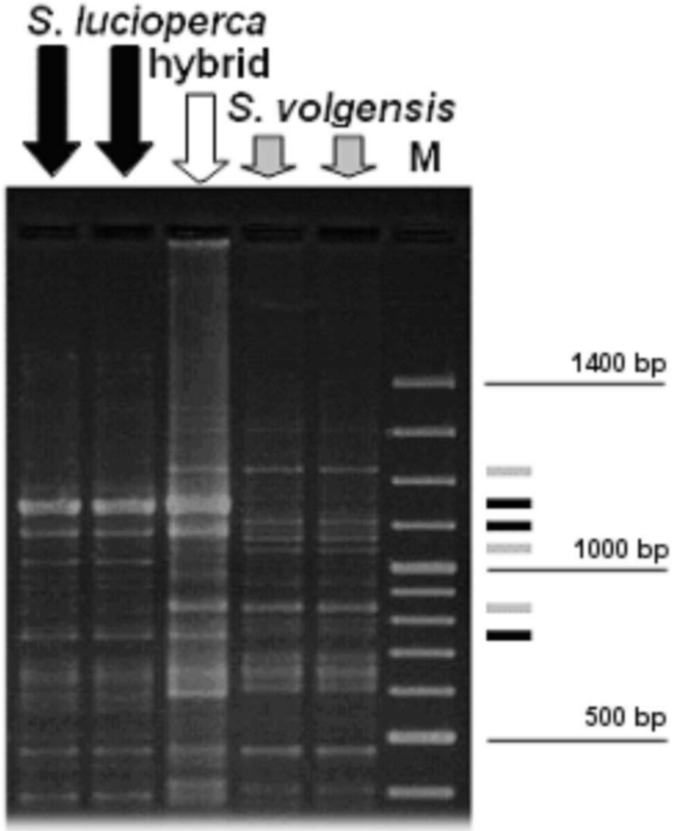


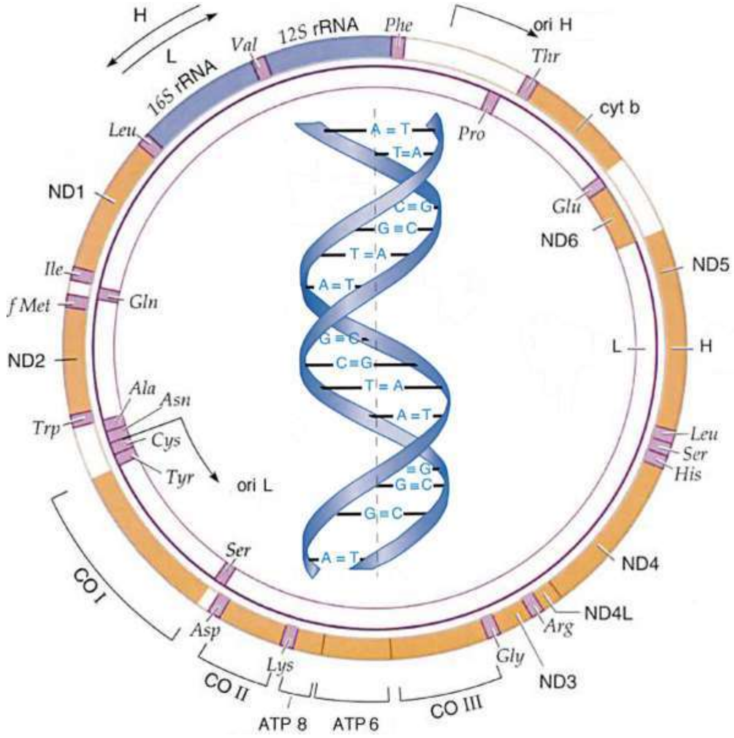


# Pike-perch X Volga sander hybrid



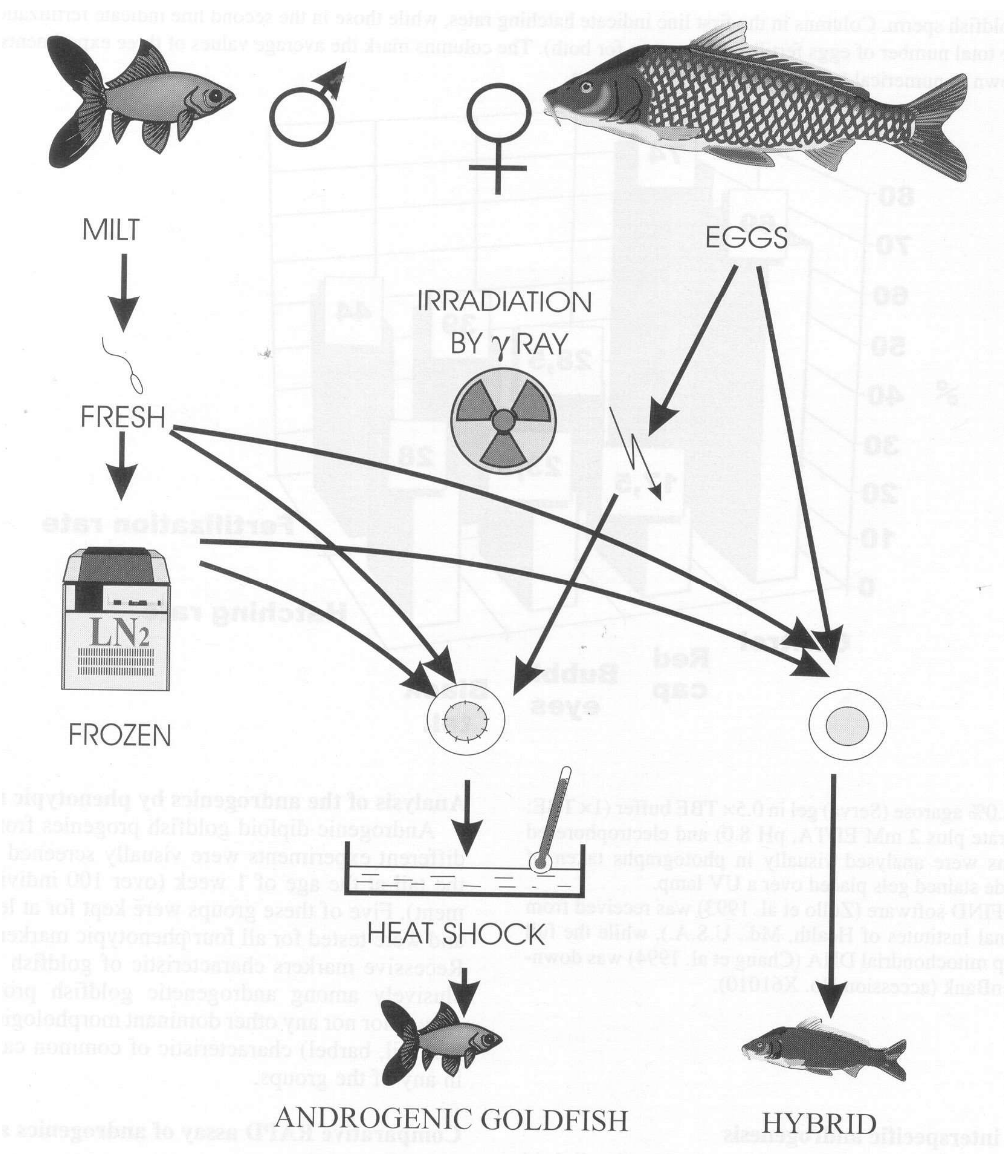
# Pike-perch X Volga sander hybrid



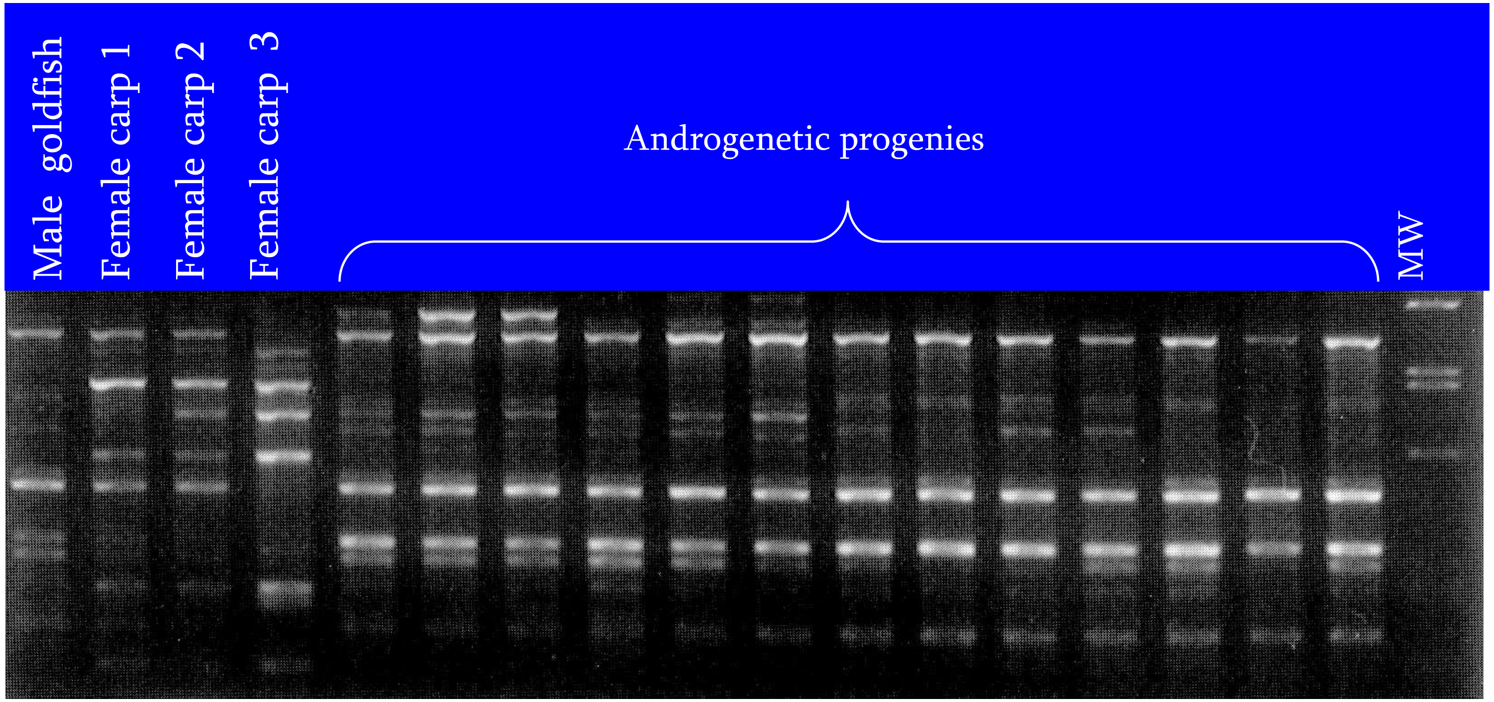


# Interspecific androgenesis

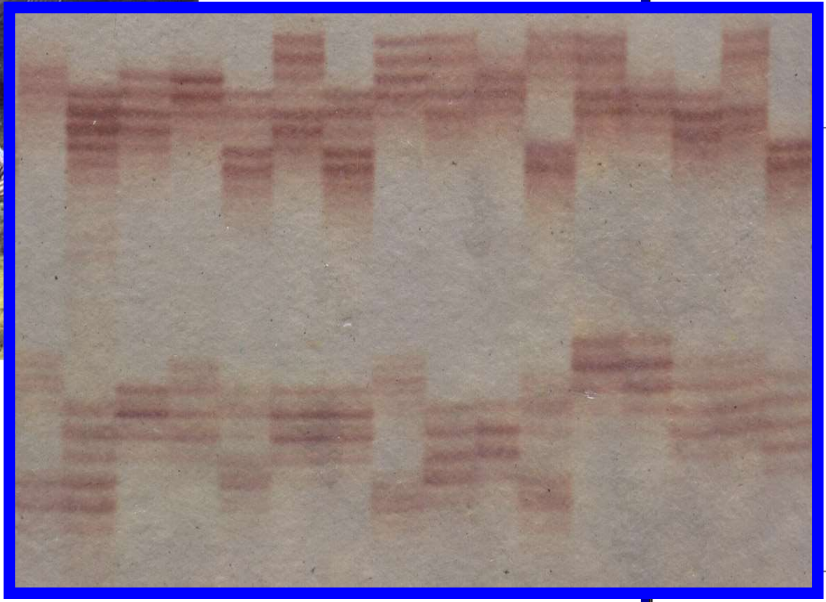
# Interspecific androgenesis

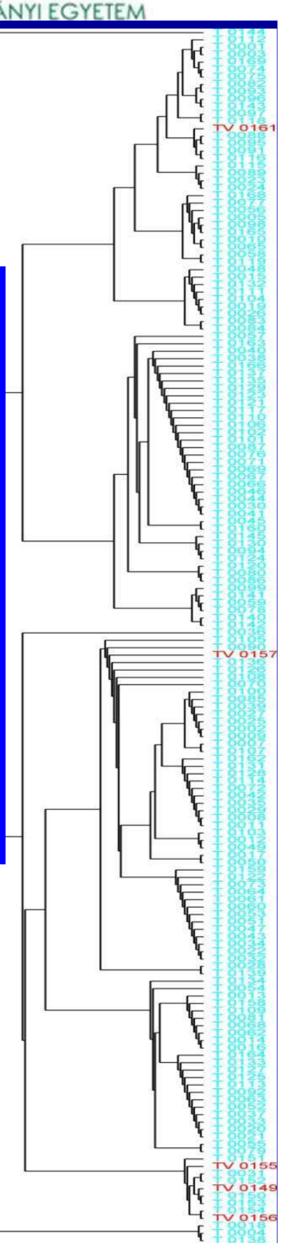


# Confirmation of interspecific androgenesis with RAPD



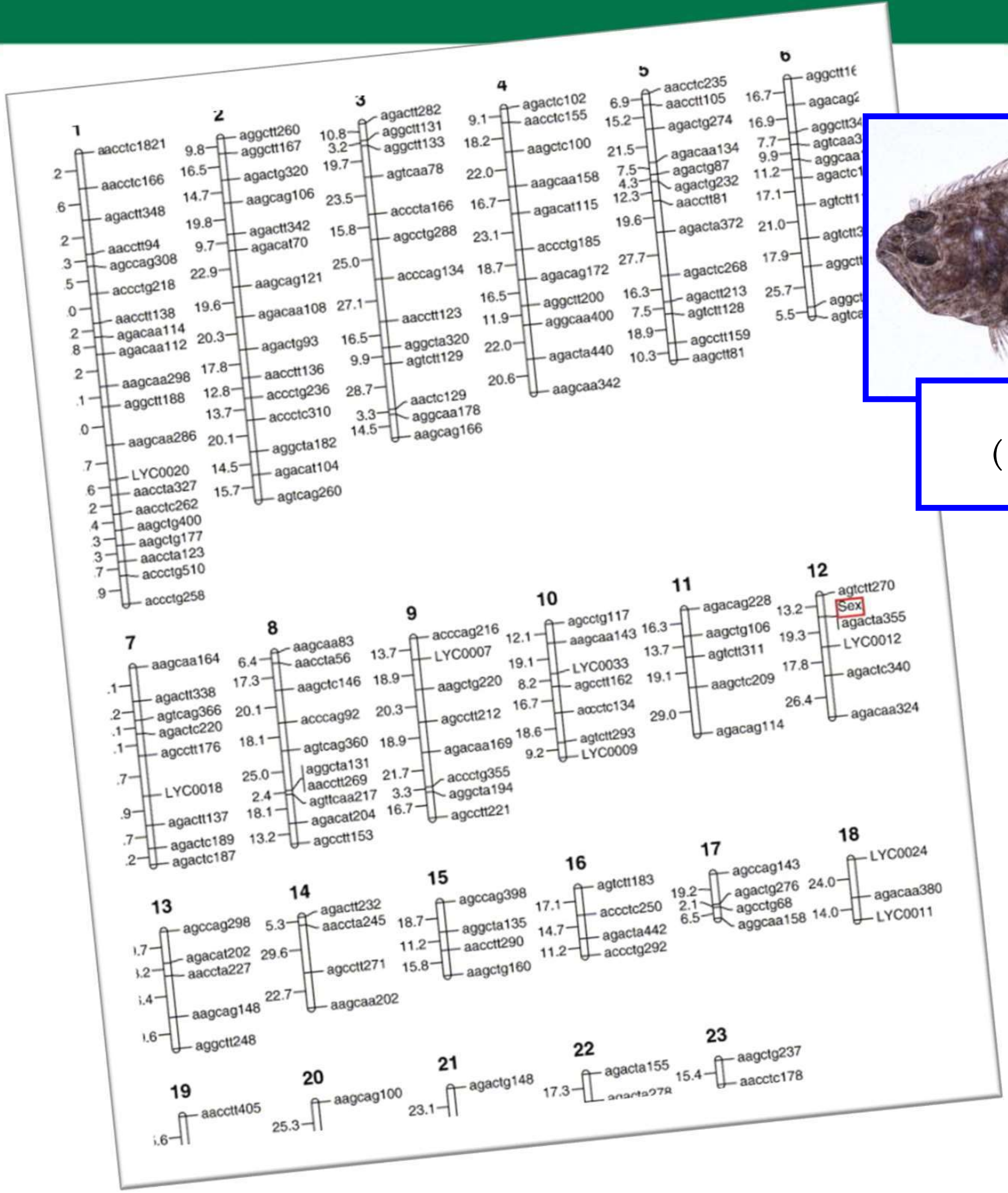
# Genetic diversity of common carp strains in Hungary

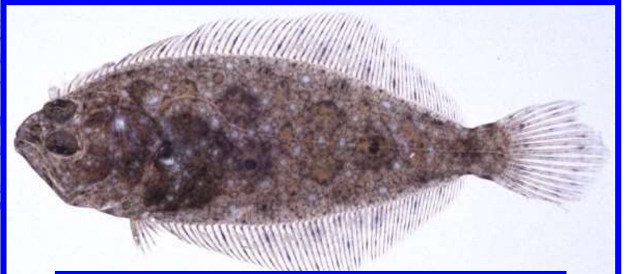




Bártfai (2003) Aquaculture 219, 1-4, 157-167
Lehoczky (2005) *Hydrobiologia* 533: 223–228

# AFLP




Yellow croaker
( *Pseudosciaena crocea)*

- 153 AFLP markers
- 94 individuals

Yue Ning 2007 Aquaculture 264, 1-4, 16-26

# Aquaculture species having genetic marker-based maps

| | |
|---|---|
| Atlantic salmon | Moen et al. (2004a)<br>Gilbey et al. (2004) |
| Rainbow trout | Nichols et al. (2003)<br>Sakamoto et al. (2000)<br>Young et al. (1998) |
| Salmonids | May and Johnson (1990) |
| Common carp | Sun and Liang (2004) |
| European sea bass | Chistiakov et al. (2005) |
| Channel catfish | Waldbieser et al. (2001)<br>Liu et al. (2003) |
| Tilapia | Lee et al. (2005)<br>McConnell et al. (2000)<br>Agresti et al. (2000)<br>Kocher et al. (1998) |
| sea bream | Franch et al (2006) |
| Japanese flounder | Coimbra et al. (2003) |
| Xiphophorus spp | Kazianis et al. (1996) |
| bufferfish | Elgar et al. (1998) |
| Cottus gobio | STEMSHORN et al. (2005) |
| walking catfish | Poompuang et al. (2004) |
| Arctic char | Woram et al. (2004) |
| Turbot | Bouza et al. (2007) |
| Guppy | Tripathi et al. (2009) |
| Zebrafish | Postlethwait et al. (1994) |
| Olive Flounder | Kang et al. (2008) |

| | |
|---|---|
| Scallop | Li et al. (2005)<br>Wang et al. (2004) |
| Pacific oyster | Hubert and Hedgecock (2004) |
| Eastern oyster | Yu and Guo (2003) |
| White shrimp | Pérez et al. (2004) |
| Kuruma prawn | Li et al. (2003) |
| Black tiger shrimp | Wilson et al. (2002) |
| Kuruma prawn | Moore et al. (1999) |
| Red Drum | Portnoy et al. (2010) |
| Lates calcarifer | Wang et al. (2010) |
| Atlantic cod | Hubert et al. (2010) |
| Grass carp | Xia et al. (2010) |
| Noble Scallop | Yuan et al. (2010) |

Over 30 species
More than 40 maps

# Known marker-QTL linked markers in aquaculture species

| Trait | Reference |
|---|---|
| **Salmonids** | |
| Spawning time | Leder, Danzmann and Ferguson (2006) |
| Early development | Martinez *et al.* (2005) |
| Pyloric caeca number | Zimmerman *et al.* (2005) |
| Natural killer cell-like activity | Zimmerman *et al.* (2004) |
| Hematopoetic necrosis resistance | Rodriguez *et al.* (2005) |
| Development rate | Sundin *et al.* (2005) |
| Infectious salmon anemia resistance | Moen *et al.* (2004c, 2006) |
| *Ceratomyxa shasta* resistance | Nichols, Bartholomew and Thorgaard (2003) |
| Infectious pancreatic necrosis resistance | Ozaki *et al.* (2001) |
| Infectious hematopoietic necrosis resistance | Khoo *et al.* (2004) |
| Body weight and condition factor | Reid *et al.* (2005) |
| Spawning date and body weight | O'Malley *et al.* (2003) |
| Growth and maturation | Martyniuk *et al.* (2003) |
| Temperature tolerance | Somorjai, Danzmann and Ferguson (2003) |
| Meristic traits | Nichols, Wheeler and Thorgaard (2004) |

| Trait | Reference |
|---|---|
| **Salmonids** | |
| Upper temperature tolerance, | size Perry *et al.* (2001, 2003) |
| Upper temperature tolerance | Danzmann, Jackson and Ferguson (1999) |
| Upper temperature tolerance | Jackson *et al.* (1998) |
| **Tilapia** | |
| Cold tolerance | Moen *et al.* (2004b) |
| Cold tolerance and fish size | Cnaani *et al.* (2003) |
| Stress and immune response | Cnaani *et al.* (2004) |
| Colour pattern | Streelman, Albertson and Kocher (2003) |
| Early survival | Palti *et al.* (2002) |
| Embryonic development | Robison *et al.* (2001) Albinism Nakamura *et al.* (2001) |
| Development rate | Nichols *et al.* (2000) |
| Spawning time | Sakamoto *et al.* (1999) |
| Sex determination | Lee, Penman and Kocher (2003); Lee, Hulata and Kocher (2004) |
| **African Catfish** | |
| Sex determination | Kovács *et al.* (2001) |

# breeding programs 1

EU funded projects:

SALMAP : new markers/maps (atlantic salmon, rainbow trout and brown trout)

SALGENE : ESTs for funcional genetics and microarray (atlantic salmon)

GRAPS : genetic mapping (atlantic salmon)
www.cgrasp.org

STRESSGENES: candidate for (MAS) Marker assisted selection (rainbow trout)
http://www.irisa.fr/stressgenes/

BASSMAP: EST and Type II marker development and mapping (Seabass)

BRIDGEMAP: Type I and Type II marker mapping (Seabream)
http://www.bridgemap.tuc.gr/

# breeding programs 2

AQUQFIRSR: identify genes associated with stress and disease resistance for MAS (fish mollusks)
http://aquafirst.vitamib.com/

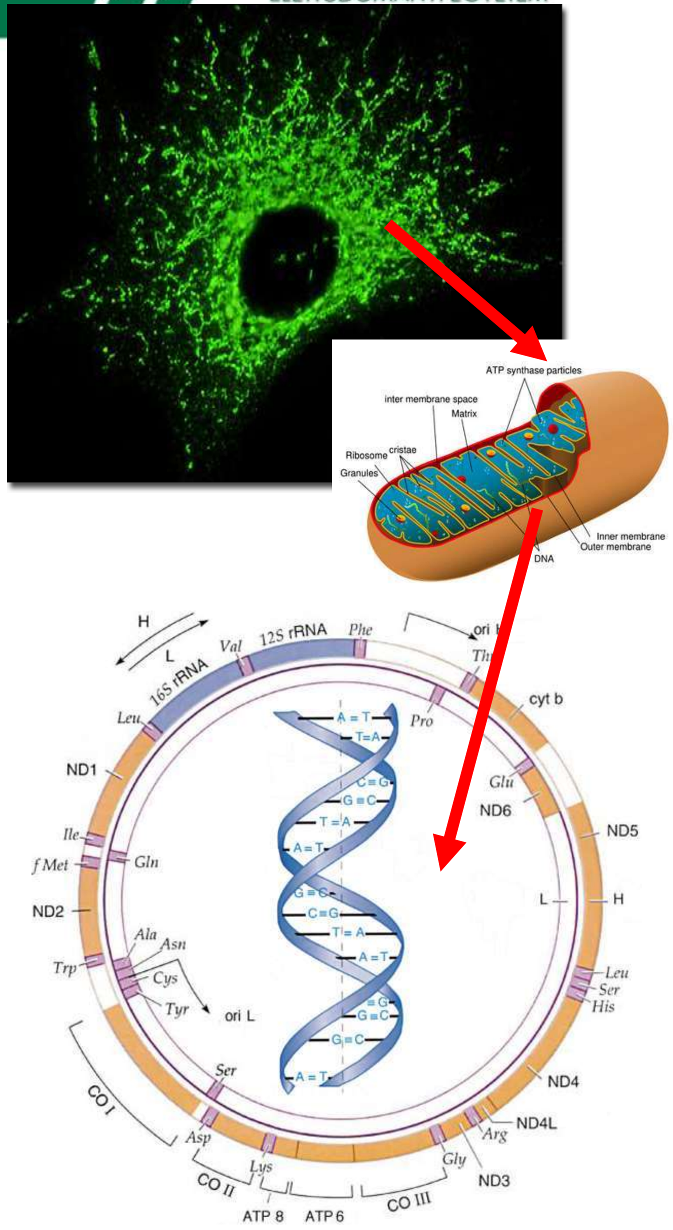
PLEUROGENE: aquaculture and genomics of two flatfish
(Halibut and Senegal sole)
http://pleurogene.ca/

CODGENE: genomics and broodstock development (Atlantic cod)
http://codgene.ca/

EUROCARP: Stress and disease resistance and broodstock development
http://eurocarp.haki.hu/

# Mitochondrial DNA

- Thousand copies / cell
- Maternal inheritance
- No recombination
- Circular DNA molecule
- Small / 16-17 Kbp
- 37 genes



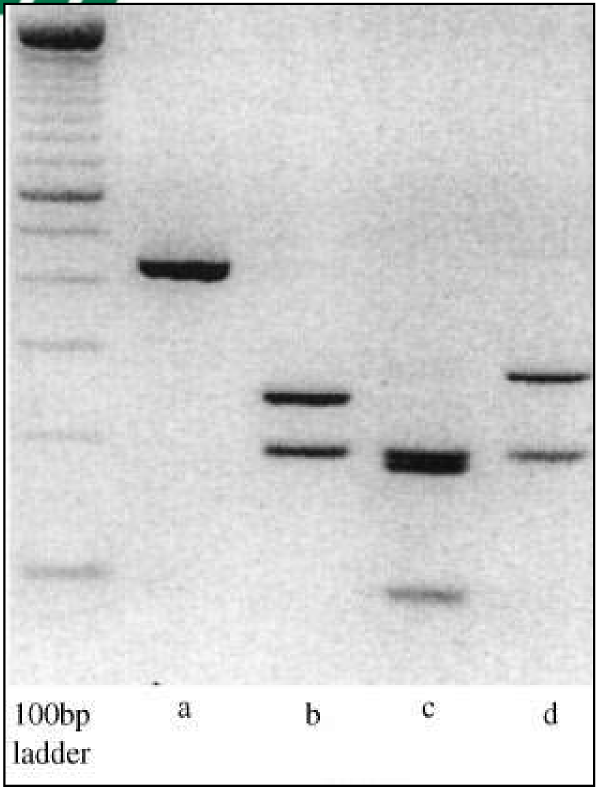
# Mitochondrial DNA

- Well conserved in vertebrates
  - No or small difference in species
  - Easy to find differences between species

- Species identification
- Evolutionary changes

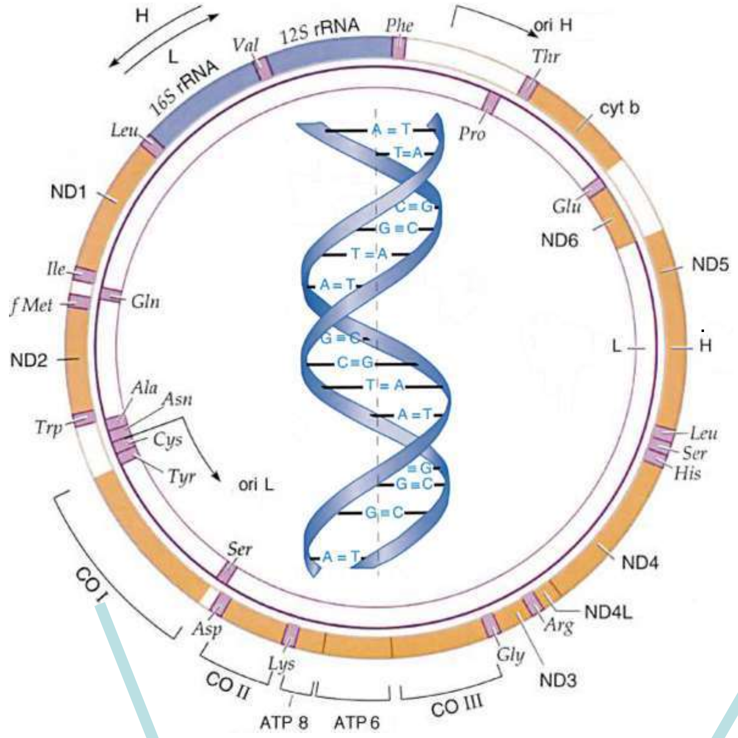
Analysis:

- PCR- RFLP
- sequencing



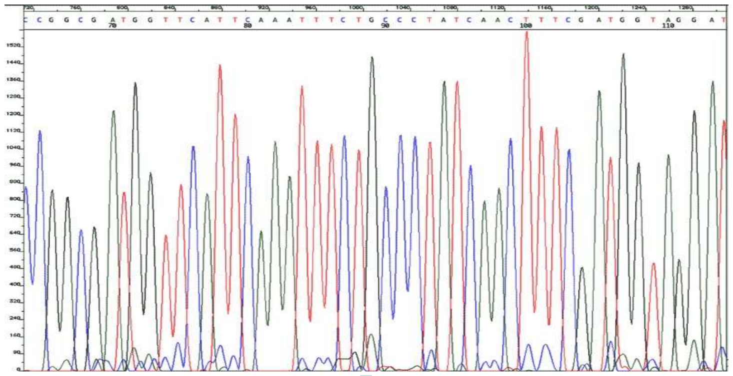
*PCR-RFLP : CytB/NlaIII*
*a: Salmo salar*
*b: Oncorhynchus kisutch*
*c: Oncorhynchus gorbuscha*
*d: Oncorhynchus keta*

*(Wolf et al. 2000)*



Cytochrome oxidase I
648bp

sequencing



Species identification

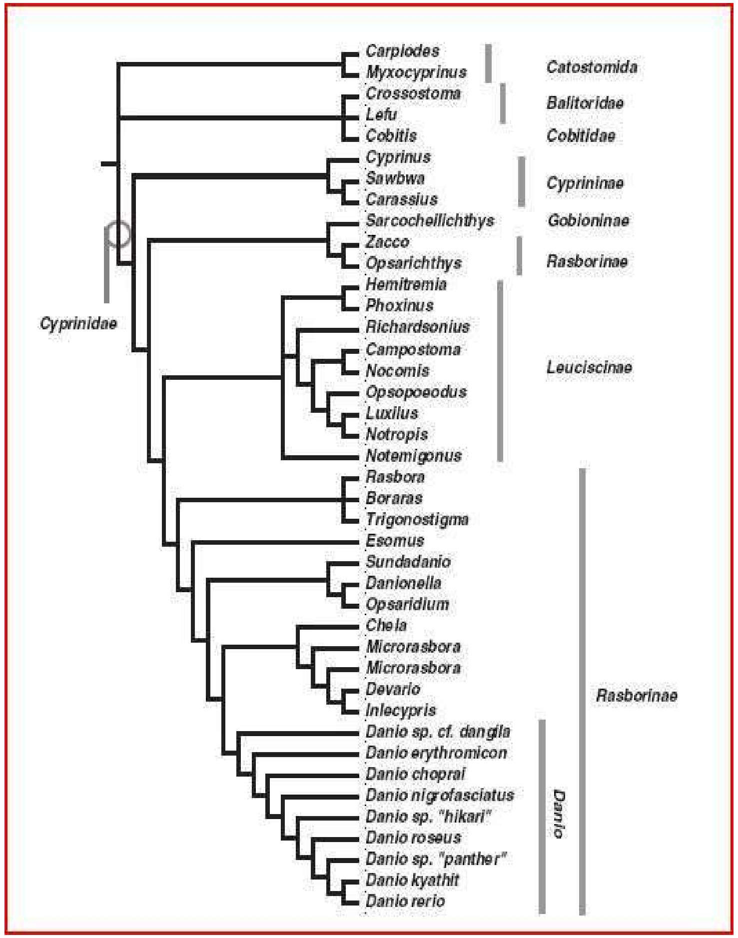
# FISH-BOL
Fish Barcode of Life Initiative

FISH-BOL

Database

Number of fish species: 31220
Barcoded fish species : 7796
(~25%)
Total number of barcodes: 56400

http://www.fishbol.org/



Taxonomy